Chapter 1

Malaria

Literature covering the history and biology of malaria, as well as the medication and treatment used in the battle against malaria.

1.1. Introduction

A disease with periodic fevers, which is thought to be malaria, has been described by writings from China from 2700 BC. Many other ancient civilised societies including the Greeks and the Romans also described these periodic fevers, with the most famous description coming from Hippocrates in the 5th century BC (Cox, 2002). Malaria was quickly associated with marshes around the city of Rome. The disease was thought to be connected to the foul smelling air from the marshes. The word malaria comes from the medieval Italian term *mal'aria* which translates to "Bad-air" and later became the word malaria, as it is used today (NIAID, 2007).

The malaria parasite was only discovered in 1880 by Charles Louis Alphonse Laveran, a French military surgeon, and in 1885, Camillo Golgi identified two different types of malaria. Both these men were awarded Nobel prizes in 1906 and 1907. Ronald Ross was the first person to demonstrate that malaria was spread by *Anopheles* mosquitoes in 1898 (CDC, 2010) for which he then received a Nobel Prize in 1902 (Amonrosa *et al.*, 2005).

Malaria control only started focusing on the eradication and control of mosquitoes after Ronald Ross's discoveries. Many new expansions into tropical areas required better control of the mosquitoes (which lead to better control of malaria as well as yellow fever). A prime example was the construction of the Panama Canal. Construction started in 1905. By 1906 of the 26 000 workers on the canal project, over 21 000 were hospitalised at some stage during their work. Many steps were then taken to stop the spread of disease. These steps included the draining of pools in the area surrounding camps, pouring oil on pools that could not be drained and keeping grass areas trimmed and jungle areas near houses deforested (Lankford, 1913). Other measures included a prophylactic dose of quinine, which was made freely available to all workers. Later all buildings were screened, to prevent mosquitoes access. By 1912 of the 50 000 people employed on the project, only 5 600 were hospitalised (CDC, 2010).

After the successful campaign in Panama, many tactics to control malaria were introduced, such as the introduction of the insecticide, dichloro-diphenyl-trichloroethane (DDT) to control mosquitoes. One of the largest success stories to come from these campaigns was the eradication of malaria from the greater United States of America as of 1951 (CDC, 2010). Campaigns as large as these are not always possible in the developing world, either logistically or monetarily. In many cases these types of projects result in creation of more problems than they solve, for instance in the early 1960's, chloroquine (one of the main antimalarials of the day) was incorporated into table salt to be distributed among the populace of Brazil in an attempt to eradicate malaria on the continent. This lead to the first reported cases of chloroquine resistance. This resistance spread at an alarming rate across the globe (Foley & Tilley, 1997).

1.2. Malaria around the world

Of the estimated global populace of 6.5 billion people, more than 1.62 billion people live in areas where malaria is endemic and more than 400 million people live in areas where there has never been any type of malaria control. This results in 350-500 million cases of malaria which are reported each year. Of these cases more than 1 million people die annually (CDC, 2010). The treatment and control of malaria is further complicated by emerging and spreading resistance to known drugs and economic problems. This makes malaria one of the most wide-spread and serious health risks in the world today.

In 2008 the WHO reported that there were an estimated 243 million (5^{th}-95^{th} centiles, 190-311 million) cases of malaria worldwide. Africa carried the biggest burden with 85% of the total. South-East Asia and the Mediterranean Regions accounted for 10% and 4%, respectively. The mortality rate of malaria paints just as bleak a picture. The total deaths caused by malaria is estimated at 863 000 (5^{th}-95^{th} centiles, 708-1003 thousand). Africa had the most deaths at 89% of the total amount of deaths (WHO, 2009).

1.3. Malaria in South Africa

Even though malaria is not as big a problem in South Africa today as it was in the past, of the estimated 40 million population of South Africa, 10% still live in malaria risk areas (Tren & Bate, 2004). The malaria risk areas in South Africa are the low-lying northern and eastern areas, or more specifically, the low altitude (below 1000m) parts of the Limpopo Province, KwaZulu-Natal, and Mpumalanga. These areas can be seen in figure 1.1. Sometimes limited outbreaks of malaria occur in the North West Province, Northern Cape and Molopo. In South Africa the risk of contracting malaria is only during the wet summer months, from October to May (DOH, 2009).

In South Africa, many different strategies have been put into use to try and limit the spread of malaria and as well as the vector mosquitoes indigenous to the area, known as *Anopheles arabiensis* and *Anopheles funestus.* Strategies included spraying with Paris Green (which was a readily accessible insecticide of the time, used from 1920 to 1946), pyrethrum insecticide and draining sites where mosquitoes breed. In 1946 the insecticide DDT came into widespread use. In certain parts of the country the incidence of malaria was reduced to $1/10^{th}$ of the original amount within 2 years. DDT is a relatively cheap insecticide which needs to be applied once or twice a year, making it even more appealing. The DDT campaign was so effective that it continued until 1996 when many environmental agencies called for a ban on DDT when it was found that DDT accumulates in fatty tissue of both human and animals (Tren & Bate, 2004).

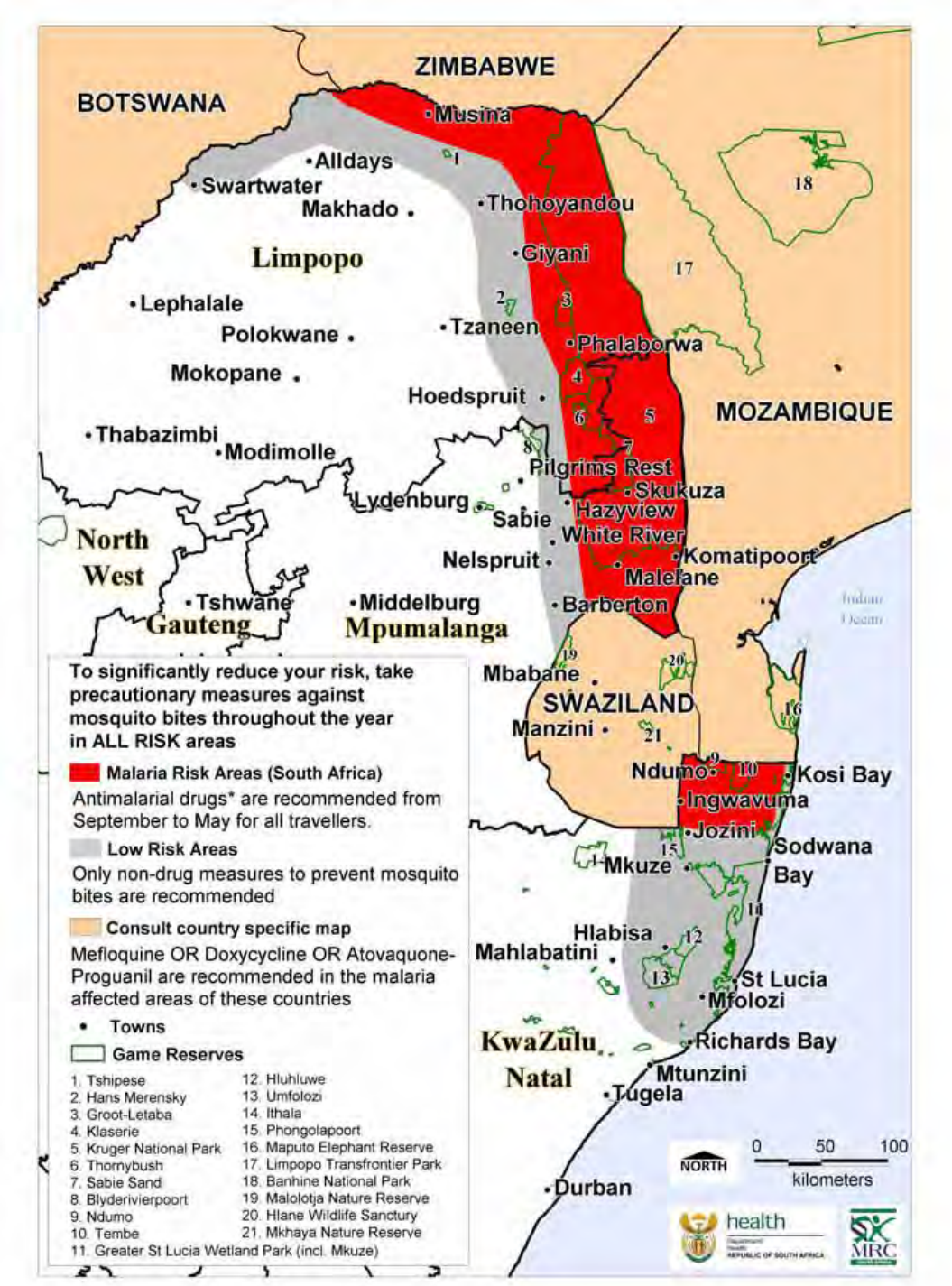


Figure 1.1: Map showing malaria risk areas in South Africa (DOH, 2009)

DDT was subsequently replaced by pyrethroid insecticides which break down faster and were deemed safer than DDT, even though no scientific study ever proved DDT to be dangerous to the environment or to humans, after more than half a century of use (Roberts *et al.*, 2000). Some species of mosquito, specifically *A. funestus* has gotten resistant to the pyrethroid insecticides. The incidence of malaria quickly escalated to worrying levels. In KwaZulu-Natal there was a 400% increase in infection rates until 1999. Other factors may have contributed to the increase, such as the level of resistance to the first line therapies of the time etc., but as

soon as DDT was reintroduced, the cases of malaria decreased by between 70% and 80% for the next three years. South Africa is one of very few African countries that has malaria under control and in some instances, has been able to contain sporadic outbreaks. This can be attributed to effective control policies, including spraying with residual pesticides, effective and current treatment regimes and adequate funding (Tren & Bate, 2004).

1.4. Biology of *Plasmodium*

Parasites of the genus *Plasmodium* are responsible for causing malaria as it is known today. Four species are known to infect humans and cause malaria, specifically *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale* and *Plasmodium malariae. P. falciparum* and *P. vivax* are responsible for most infections worldwide with *P. falciparum* responsible for the most severe symptoms as well as deaths annually (CDC, 2010). Deaths resulting from the other species are quite uncommon (Merck Research Laboratories, 2008). More than a hundred species of *Plasmodium* exist, such as *Plasmodium yeolii* and *Plasmodium berghii,* which are confined to other non-human mammals (Tripathi *et al.*, 2005). Some of these species are used in continued malaria research programmes. These species cannot infect human hosts under normal circumstances (Tuteja, 2007).

Malaria is spread by female *Anopheles* mosquitoes. These mosquitoes carry the malaria parasites in their salivary glands in the sporozoite form. The life cycle of Plasmodium can broadly be split into two stages, the sexual stage and the asexual stage, as can be seen in figure 1.2. The sexual stage occurs inside a mosquito host and the asexual in a human host. The asexual stage of the lifecycle can be divided into the phase outside erythrocytes also known as exo-erythrocytic stage and the phase inside erythrocytes or erythrocytic stage (Wiser, 2003).

1.4.1. Asexual stage

When the infected female *Anopheles* mosquito takes a blood meal from a human host, the mosquito pierces the skin with elongated mouthparts and injects its saliva into the wound. The saliva contains an anticoagulant as well as the malaria parasite in the sporozoite form. From the site of injection the sporozoites enter into the blood circulation and travel to the parenchiem cells of the liver. The time needed to travel from the site of infection to the liver depends on the specific type of malaria.

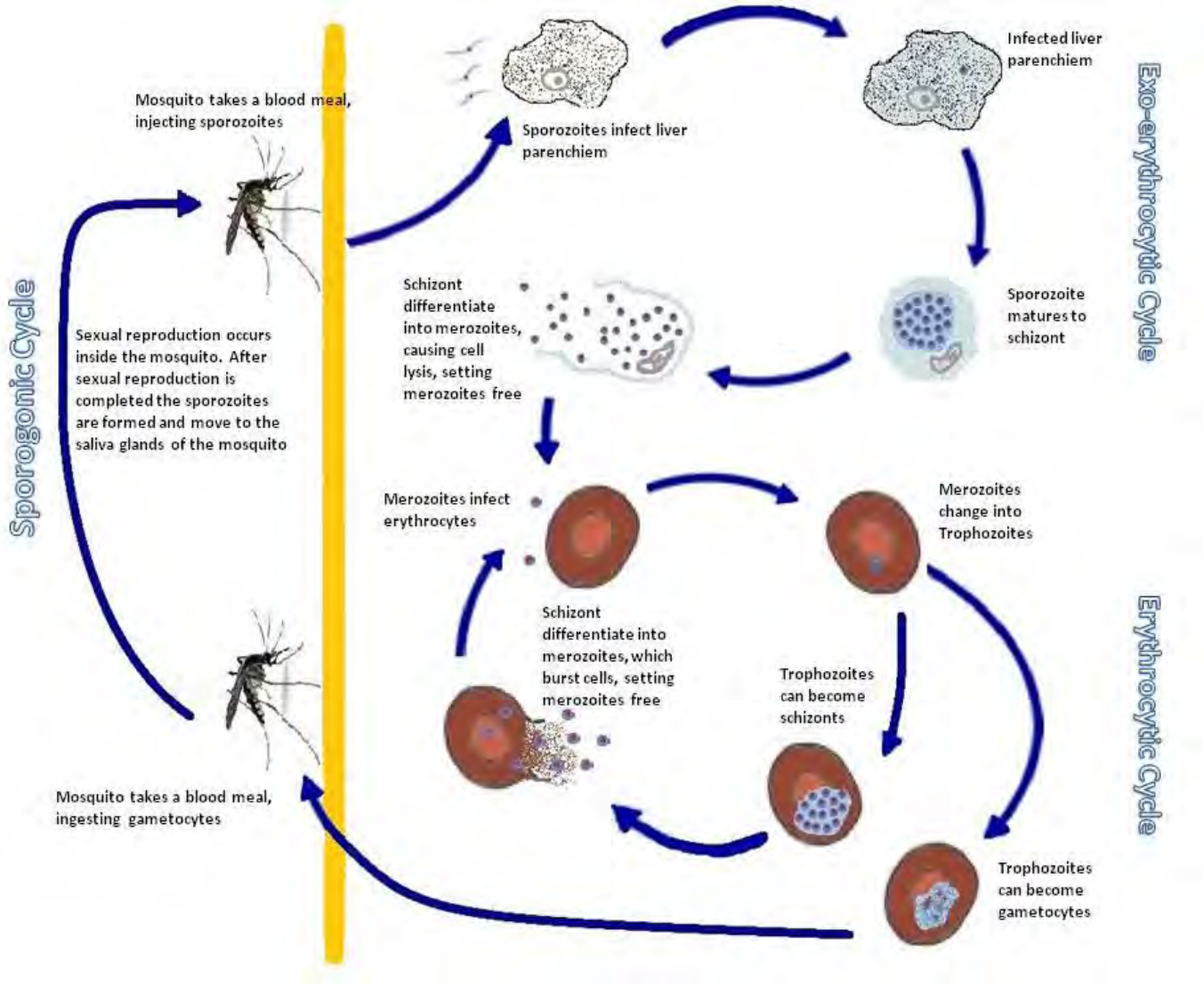


Figure 1.2: A schematic representation of the lifecycle of *P. Falciparum* (Adapted from CDC, 2010; Rosenthal, 2004)

In the liver the sporozoite infects the liver cells which replicate mitotically. This process is known as pre-erythrocitic schizogony. The process by which the sporozoites enter the parenchiem is still highly debated (Baum *et al.*, 2008). This is important to know, as it may be a target for new drugs or even a malaria vaccine (Walgate, 2001). The sporozoite matures into a schizont which then differentiates into merozoites. The merozoites are released into the bloodstream. Certain species of *Plasmodium* specifically *P. vivax* and *P. ovale* are able to keep a few of the sporozoite in a dormant state or a hypnozoite. The hypnozoite stage is mostly responsible for relapsing bouts of malaria, months or even years after the initial infection has taken place (Shapiro & Goldberg, 2006; Wiser, 2003).

The free merozoites are able to infect erythrocytes in a process called erythrocitic schizogony. The process consists of four stages. Once inside the erythrocytes, merozoites changes to trophozoites. This is the beginning of the so-called ring phase. In the beginning of this phase the

trophozoite absorbs a lot of the host cells cytoplasm into its own digestive vacuole (Foley & Tilley, 1997). This makes the digestive vacuole very prominent, which gives the ring like appearance on microscope slides. The vacuole shrinks as time passes (Baum *et al.*, 2008).

The trophozoite then changes into a schizont. Inside the schizont up to 20 merozoites are formed. The infected red blood cells then undergo cell lysis, releasing the newly formed merozoites, which can reinfect new red bloods cells. The release of merozoites is also a possible site for drug interference in the parasite lifecycle, even if it is just to decrease the severity of disease (Walgate, 2001). When the red blood cells burst, not just merozoites are released into the blood stream, but many other waste products. This is one of the main factors responsible for the characteristic fever with which malaria presents. Later on, a small number of merozoites develop into macro- and microgametocytes, which are necessary for the parasite to reinfect mosquitoes (Wiser, 2003). In this form the parasites are able to sexually reproduce (only inside the mosquito host), but this form of the parasite is not alive for very long and is completely inactive in a human host (Rosenthal & Katzung, 2004).

1.4.2. Sexual stage:

This stage is also known as the sporogonic cycle as illustrated on figure 1.2. When the *Anopheles* mosquito takes a blood meal from an infected human host, the gametocytes may be ingested. These gametocytes form micro- and macrogametes. The microgametes are sperm-like and can fertilise the macrogametes, which then form a zygote. The zygote develops into an ookinete, which can penetrate the cell membrane of the midgut, from where it can create thousands of sporozoites, which reach maturity in ten to fourteen days. The sporozoites travel to the salivary glands, from where the next host can be infected, thus the lifecycle is completed. The completed sexual stage takes between 10 and 18 days (CDC, 2010).

1.5. Clinical appearance of malaria

The time it takes for malaria symptoms to appear is dependent on the species of *Plasmodium* with which the person was infected. The incubation times are as follows:

- 9 to 14 days for *P. falciparum*.
- 12 to 17 days for *P. vivax*.
- 16 to 18 days for *P. ovale*.

- 18 to 40 days for *P. malariae.*

It is important to note that *P. vivax* and *P. malariae* may be latent for years before showing any symptoms (Pearson, 2009). The symptoms for malaria infections are basically the same for all the different types. The severity of symptoms may differ vastly from species to species and depend on the infected person's immunity to malaria. In areas where patients are exposed to malaria on a regular basis, partial immunity may develop. These patients often do not show any acute symptoms (WHO, 2009). The basic symptoms are fever, rigor, headaches, fatigue, anaemia, jaundice, splenomegaly or hepatomegaly, vomiting and joint pain (Rosenthal, 2004; Pearson, 2009; CDC, 2010). These symptoms are mostly connected to uncomplicated malaria (Wiser, 2003; DOH, 2008).

The malaria paroxysm as it is known, starts with a general feeling of being unwell with chills. In this time, fever may rise to between 39 to 41 ºC. Other symptoms that appear are muscle pain, headaches, nausea, polyuria and quickened pulse. After the fever has subsided, profuse sweating occurs. This is usually followed by intense fatigue. The fever occurs when erythrocytes rupture (Rosenthal, 2004), releasing the merozoites as well as toxins, which in turn trigger the release of cytokines, which cause fever and chills (Wiser, 2003).

Severe malaria occurs when uncomplicated malaria goes untreated or if there is a treatment failure. The WHO defines severe malaria as presenting with any of the following symptoms, when all other causes of the symptoms have been eliminated:

- Impaired consciousness or any change in level of consciousness.
- Prostration (Patient is too weak to move without assistance).
- Failure to eat.
- Convulsions (More than once in a 24 hour period).
- Respiratory distress.
- Going into shock.
- Apparent dysfunction of any vital organs.
- Spontaneous bleeding.
- Pulmonary oedema (WHO, 2009).

P. falciparum is responsible for the most severe symptoms as well as annual deaths (CDC, 2010). *P. falciparum* is one of the only strains of malaria that is likely to cause death when left untreated, with death occurring within days of the first symptoms appearing. Deaths resulting from the other species are quite uncommon (Pearson, 2009).

1.6. Drug resistance

The efficacy of any antimalarial is based on how effective the drug is in killing parasites. In other words, it is the ability of the parasite to survive a drug therapy, even after drug dosages have been pushed higher than regular doses. Resistance develops when drugs are used incorrectly or when drugs are used without proper control (WHO, 2009). In certain cases resistance to antimalarial drugs develops due to gene mutations, independent of the effect that the drug has (Wongsrichanalai *et al.*, 2002).

Within the last few decades, resistance to all known antimalarials have developed, even for the newly introduced artemisinin derivatives (WHO, 2009; Dondorp *et al.*, 2009). The time it takes for resistance to develop is relatively short as can be seen in table 1.1.

Table 1.1: Introduction dates of specific antimalarial drugs and time taken for the appearance of resistance (adapted from Wongsrichanalai *et al.*, 2002)

Drug	Introduction year	Appearance of resistance	Time taken for resistance to appear (years)
Quinine	1632	1910	278
Chloroquine	1945	1957	3
Proguanil	1948	1948	2.4
Sulphadoxine-pyremethamine	1967	1967	2.4
Mefloquine	1977	1982	5
Atovaquone	1996	1996	6

Drug treatment policies need to be reviewed on a regular basis. This ensures that any mortality and morbidity which can be linked to malaria will be decreased, as well as ensuring that current treatments work effectively and no recrudescence of infections occur (WHO, 2009). Resistance to antimalarial drugs are not just confined to *P. falciparum*. Other strains have been documented as resistant, for example*, P. ovale*, *P. vivax*, and *P. malariae*. In other cases *P. falciparum* has become resistant to more than one drug, thus becoming part of the multi-drug resitant strains (Wongsrichanalai *et al.*, 2002). The WHO prescribes that malaria treatment should be changed as soon as treatment failure reaches 10%. One of the best methods of stopping or, at least slowing down resistance is to combine two antimalarials that have different mechanisms of action. Monotherapy methods that have been used in the past are strongly discouraged (WHO, 2009).

1.7. Malaria treatment: South Africa treatment regimes

The South African Department of Health released the standard treatment regime for malaria cases in South Africa in the Standard Treatment guidelines and Essential Medicine List (2008 edition) as well as in the Nationally released Guidelines for the treatment of Malaria in South Africa. The regime includes treatment options for uncomplicated *P. falciparum,* severe *falciparum* infections, as well as non-*falciparum* infections.

1.7.1. Treatment of uncomplicated *P. falciparum* Malaria

Uncomplicated *P. falciparum* malaria is defined as mild symptoms and the patient is still able to walk around unaided, no signs of organ failure is present and the parasitemia in measured blood samples (either clinical or laboratory tests) should be less than 5% (WHO, 2009; DOH, 2008). The South African Medicines formulary (SAMF) describes uncomplicated malaria as a patient that is ambulant, has a normal mental state, adequate urine output and is not vomiting repeatedly (Rossiter, 2009). The treatment regime for the treatment of uncomplicated malaria in South Africa can be seen in table 1.2. This is the regime as prescribed by the DOH in 2008.

Table 1.2: Treatment regime for uncomplicated *P. falciparum* malaria (DOH, 2008)

	Recommended treatment	Alternate treatment
Adult	Arthemether-lumefantrine fixed dose combination (Coartem®) given with a fat containing meal	Quinine combined with doxycycline for seven days. Caution needs to be taken with this treatment. Treat patients as inpatients
Paediatric (Children older than a year)	Arthemether-lumefantrine fixed dose combination (Coartem®) given with a fat containing meal.	Quinine combined with either doxycycline or clindamycin after two to three days of quinine treatment. • Quinine is given as an oral dose. • Younger than 8 years use clindamycin. • Older than 8 years use doxycycline.
Pregnancy	Quinine combined with either clindamycin after two to three days of quinine treatment.	Give artemisinin derivatives (in combination with lumefantrine) only in second and third trimesters if there are no alternatives available.

Since 2006 many countries have changed their first line treatment strategies from the chloroquine (CQ) and sulfadoxine-pyrimethamine (SP) combination to the artemisinin-based combination therapies (ACT's) because of rising chloroquine resistance. This process is slow to occur as the new drugs are expensive. The WHO has recommended the use of the combination of amodiaquine and sulfadoxine-pyrimethamine to treat uncomplicated malaria in the Guidelines for the Treatment of Malaria (2009) (DOH, 2009) as an alternative to the more expensive ACT's, but this is only for areas where amodiaquine resistance has not been documented (WHO, 2009).

1.7.2. Treatment of severe *P. falciparum* malaria

Uncomplicated malaria can quickly deteriorate to severe malaria which increases the morbidity and mortality rates. When treating uncomplicated malaria extra precaution needs to be taken with children, pregnant women, immune-suppressed patients and non-immune persons. Patients suspected to have severe malaria must be moved to high care facilities. Supportive care is of the utmost importance with regular monitoring of blood glucose levels, renal function and blood oxygen saturation.

Severe malaria is defined as a patient with a parasitemia of ≥ 5%, with any or all of the following symptoms: complete physical exhaustion, impaired consciousness, problems breathing (acidotic breathing), bleeding tendencies, pulmonary oedema, shock (blood pressure dips below 70mm Hg in adults and below 50mm Hg in children), convulsions, haemoglobinuria, decrease in urine output and any other signs of organ failure (DOH, 2009; WHO, 2009).

As in other treatment schemes, it is of utmost importance that health care practitioners must be aware of any possible side-effects and be prepared for any and all adverse reactions to the antimalarial that is being put into use, especially when treating severe malaria. Treatment measures need to be put into place in case of complications. Complications include: anaemia, hypoglycaemia (which is quite common and to be expected when treating with quinine), cerebral malaria, renal failure, hepatic dysfunction, shock, metabolic acidosis, circulatory collapse and respiratory complications (especially in pregnant patients or patients with existing respiratory problems (DOH, 2008; Rossiter, 2009). The treatment regime for the treatment of uncomplicated malaria in South Africa can be seen in table 1.3. This is the regime as prescribed by the DOH in 2008.

Table 1.3: Treatment regime for severe *P. falciparum* malaria (Department of health, 2008)

	Recommended treatment	**Alternate treatment**
Adult	Quinine given as slow intravenous infusion (Never by bolus injection). • After 48 hours the patient should be able to take quinine orally. • After improvement clindamycin or doxycycline should be added to the treatment.	In cases where quinine resistance is suspected or side-effects that present are too severe. • Parentaral artemisinin derivatives such as arthemether or arthesunate should be considered (Not registered in South Africa yet).
Paediatric	Quinine given as slow intravenous infusion (Never by bolus injection). • This treatment must be combined with either doxycycline (if the patient is older than 8 years) or clindamycin (if the patient is younger than 8 years of age).	Artemisinin derivatives have become known to be safe alternatives but no intravenous or rectal treatments have been registered for use in South Africa (same as for adult treatment).
Pregnancy	Quinine given as slow intravenous infusion (Never by bolus injection).	Artemisinin derivatives have become known to be safe alternatives but no intravenous or rectal treatments have been registered for use in South Africa (same as for adult treatment).

1.7.3. Treatment of Non-*P. falciparum* infections

It is of utmost importance to confirm the diagnosis of any *Plasmodium* species infecting a patient with the use of microscopy methods. This must be done to ensure that the specific species of Plasmodium is treated, as treatment courses differ for different species. If the diagnosis is unsure, the standard treatment for *P. falciparum* should be started. In the case of a pure *P. malariae* infection, a chloroquine monotherapy is indicated. *P. vivax* and *P. ovale* can also be treated with either chloroquine, quinine or arthemether-lumefantrine, but a follow up treatment of primaquine is indicated to treat any hepatic phase of the parasite that may still be present, and in so doing prevent any chance of relapse (WHO, 2009; DOH, 2008).

The use of primaquine in children (younger than 1 year), pregnancy and severe G6PD (glucose-6-phosphate dehydrogenase) deficiency is contra-indicated (non-severe G6PD deficiency may use primaquine, if patient is closely monitored). If a mixed infection (i.e. *P. falciparum* with any other type of *Plasmodium*) is suspected, the standard treatment for *P. falciparum* should be followed by a course of treatment using primaquine (DOH, 2008).

1.8. Antimalarial drugs

As described earlier, many ancient civilisations identified malaria-like symptoms. Many of these writings described treatment methods. Writings from China dated to the second century BCE described a plant called Quinhao (*Artemisia annua L*. also known as sweet wormwood) which was used in the treatment of malaria. The active ingredient was later found to be artemisinin by Chinese scientists in 1971 (CDC, 2010). Artemisinin is a big part of modern malaria treatment therapies. It is most often given in combination with other antimalarials (WHO, 2009).

1.8.1. Classification of antimalarial compounds

The classification of antimalarials can be made according to many different systems. The chosen system uses the stage in the lifecycle where the antimalarial employs its effect. The system is as follows:

- **Sporontocides:** Prevents the sexual stage in the replication of malaria parasites inside the mosquito.

- **Tissue schizontocides**: These antimalarials prevent the schizonts from entering and replicating inside the erythrocytes.
- **Blood schizontocides:** The antimalarial effect takes place when the parasite is inside the erythrocyte.
- **Gametocytocides**: These drugs eliminate the gametocytes from the host circulation, thus preventing the parasites from infecting mosquitoes (Sweetman, 2009).

The quinoline antimalarials are specifically known for their efficacy as blood schizontocides.

1.9. Quinoline antimalarials

The discovery of the Americas led to the discovery of one of the most effective malaria cures yet. It was discovered that Peruvians have long since been using the bark of the cinchona tree to cure fevers (CDC, 2010). Cinchona bark was then systematically transported to Europe by Jesuits. The powdered bark has been used since the early 1600's in many different forms, curing many people, even European royalty. It was not until 1820 that Pierre-Joseph Pelletier and Joseph Bienaimé identified the pharmacological active in the cinchona bark and named it quinine. Unfortunately, supply often was not large enough to meet demands for the bark, especially at times of war. Therefore, the quest to create synthetic quinine started in 1850. Only in 1944 was the molecular structure of quinine identified by Woodward (Woodward & Doering, 1945). Synthetic production of quinine was still not viable as problems with the stereochemistry made commercial production of the molecule impossible for many years (Soloman & Lee, 2009).

The search for a synthetic antimalarial was initiated when the Paul Ehrlich group discovered in 1891 that methyl blue (a synthetic dye) had some antimalarial activity. It was later found that with replacement of one of the methyl side chains, the antimalarial activity increased. This led to the development of plasmoquine (also known as pamaquine) in 1926 by German scientists working in Bayer laboratories of IG Farben. This first antimalarial which is known as an 8-aminoquinilone was very effective but also extremely toxic, thus it was removed from use (Soloman & Lee, 2009).

1.9.1. Mechanism of action

The mechanism of action for 4-amino-quinoline compounds (like quinine, chloroquine and amodiaquine) are still largely uncertain, with many hypotheses competing for the top spot. It has

been found that the mechanism of action is strongly dependent on the accumulation of the drug in the digestive vacuole of the parasite (Ginsberg *et al.*, 1999). The precise mechanism by which the quinoline antimalarials induce schizontocidal action is highly debated with the following mechanisms being the main theories.

1.9.1.1. DNA intercalation

The 4-aminoquinolines have become known for their strong interactions with DNA and RNA. Chloroquine (CQ) has become specifically known for this effect. Chloroquine is suggested to form complexes with DNA, thus, creating defects in the DNA. This leads to further problems in the replication of DNA strands (Soloman & Lee, 2009). However, this effect does not seem to be connected to the antimalarial activity (Foley & Tilley, 1997).

1.9.1.2. Inhibition of haemoglobin degradation

The quinoline antimalarials are specifically active in the mature schizont phase of the lifecycle of *Plasmodium*. In this phase the parasite needs to ingest haemoglobin from the cytosol of the host erythrocyte. It is, therefore, thought that the drugs interfere with either the uptake or the digestion of haemoglobin. When haemoglobin is ingested by endocytosis it is transported to the food vacuole. The quinoline antimalarials, especially chloroquine and amodiaquine are specifically known to concentrate in the food vacuole. When these antimalarials are added to infected erythrocytes, the food vacuole of the parasite swells with undigested haemoglobin (Foley & Tilley, 1997).

1.9.1.3. Haem polymerisation theory

The parasite uses its food vacuole to digest haemoglobin which is found in cytoplasm that was absorbed from the host erythrocyte. Chloroquine and the other 4-aminoquinolines also get absorbed into the food vacuole of the parasite. The digestion of haemoglobin produces ferriproporphyrin IX (FP) as a metabolite. FP is a toxic substance which can bind to enzymes, inhibiting their action, or even damage cell membranes to the point where cell lysis takes place. The parasite is able to convert the toxic FP to hemozoin, which is harmless (Warhurst, 1995). It is thought that chloroquine and amodiaquine, which concentrate inside the food vacuole of the parasite form a complex with the FP. This complex cannot be polymerised to the hemozoin form. The FP is free to leave the food vacuole. The FP is able to bind to the cell membranes,

causing oxidative stress, which in turn causes cell lysis or interferes with other enzymes, inhibiting their functions (Sullivan *et al.*, 1998; Becker *et al.*, 2004).

1.9.1.4. Integrated model

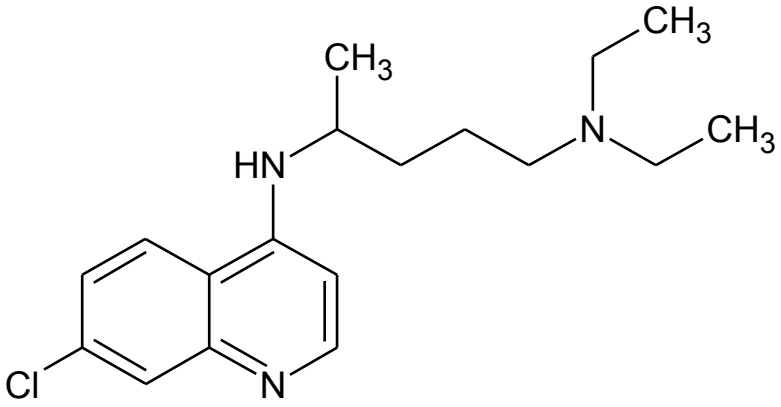
Another theory which has been put forward by Ginsberg *et al.* (1999) stated that although FP is polymerised, FP would escape into the cytosol of the parasite regardless of being polymerised or not, as FP is not completely polymerised. It has been found that FP can be broken down by free gluthatione (GSH) in the cytosol of the parasite, oxidising the gluthatione and forming gluthatione disulfide (GSSG). The GSSG is then reduced back to GSH by gluthatione reductase (GR) with the help of energy supplied by NADPH. It is theorised that the quinoline antimalarials bind to the GSH, inhibiting its action, thus freeing the FP to attach to cell membranes which causes cell lysis by way of oxidative stress (Ginsberg *et al.*, 1999).

1.10. Chloroquine

After the initial discovery of plasmoquine, German scientists continued experimenting with the structure thereof in an effort to increase efficacy as well as decreasing toxicity. This led to the synthesis of the acrinine derivative, which is also known as quinacrine, atebrine or mepacrine. From these structures, Andersag *et al.* (1942) discovered two 4-aminoquinoline structures, known as sontoquine and SN 3294, which then led to the discovery of resochin. Resochin was initially thought to be too toxic for human consumption, but with further investigation it was found to be safe and effective at therapeutic concentrations. The development of chloroquine's, structure is depicted in Figure 1.3. Resochin was renamed to chloroquine and released on the market, where it was one of the most successful antimalarials to date, but instances of chloroquine resistance were first reported in the early 1960's after huge population-based treatment campaigns in Brazil. The resistant strain started to spread at a remarkable pace and is now a worldwide occurrence (Foley & Tilley, 1997). Table 1.4 shows the physical properties of chloroquine.

1.10.1. Properties

Table 1.4: The physical properties of chloroquine (United States Pharmacopeia, 2010)

Chemical composition	Mannich-base 4 Amino-quinoline derivative
Structure	$C_{18}H_{26}ClN_3$
The chemical structure of chloroquine	
Chemical name	7-chloro-4-(4'-dethylamino-1'-methylbuthylamino)quinoline
Formulary weight	319.9 g/mol
Description	• White crystalline powder • Practically insoluble in alcohol
Solubility for sulphate and phosphate salt forms	• 1g dissolves in 3-4 ml of water • Practically insoluble in alcohol
Type of antimalarial	Blood schizontocide

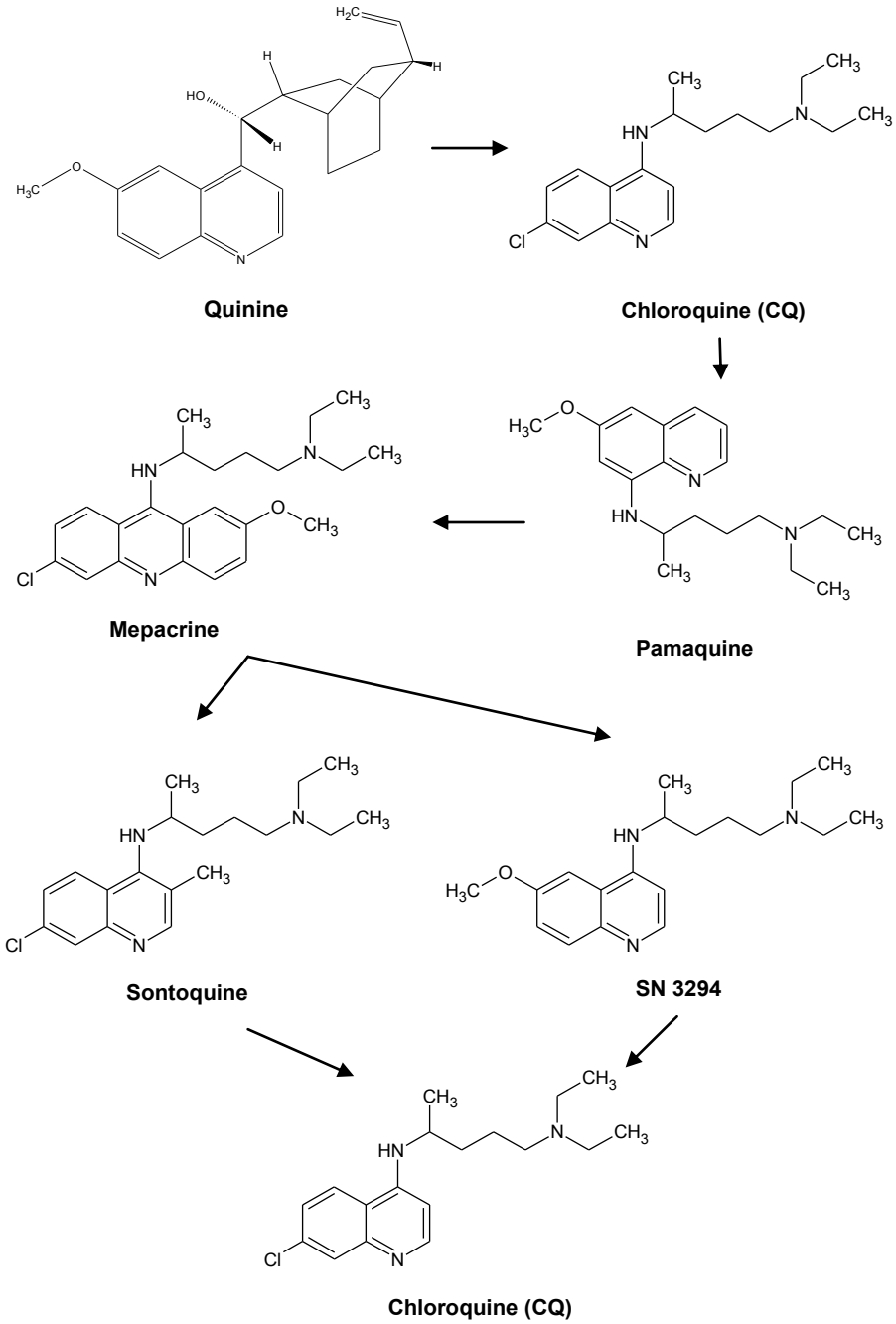


Figure 1.3: The development of chloroquine from quinine (Soloman & Lee, 2009)

1.10.2. Pharmacokinetics

When taken orally, chloroquine is absorbed rapidly, reaching the maximum plasma concentration within 3 hours. Chloroquine has a huge volume of distribution (approximately 100-1000 l/kg) and distributes to many tissues that release it slowly. The released chloroquine is metabolised to mono-desethylchloroquine in the liver and is excreted via the kidneys (WHO, 2009). According to Bergqvist *et al.* (1983), chloroquine concentrates in erythrocytes contaminated with malaria, but in uninfected blood, only 12% of the chloroquine collects in

erythrocytes, 36% in plasma and the rest in the leukocytes and platelets (Bergqvist & Domeij-Nyberg, 1983).

1.10.3. Side-effects

In some people chloroquine can produce serious pruritus or itching, which is a very common side-effect. Other side-effects include nausea, vomiting as well as cramps. Transient headaches are less common but do appear. Other very rare effects include: bleaching of the hair, polyneuritis and ototoxicity. When used for long periods, chloroquine may accumulate in the retina which can cause permanent sight damage. This only occurred in cases where dosages for prophylaxis were exceeded for very long periods.

Chloroquine has been contraindicated for persons with epilepsy, persons who have a known hypersensitivity as well as persons suffering from psoriases (Rosenthal & Katzung, 2004).

1.11. Amodiaquine

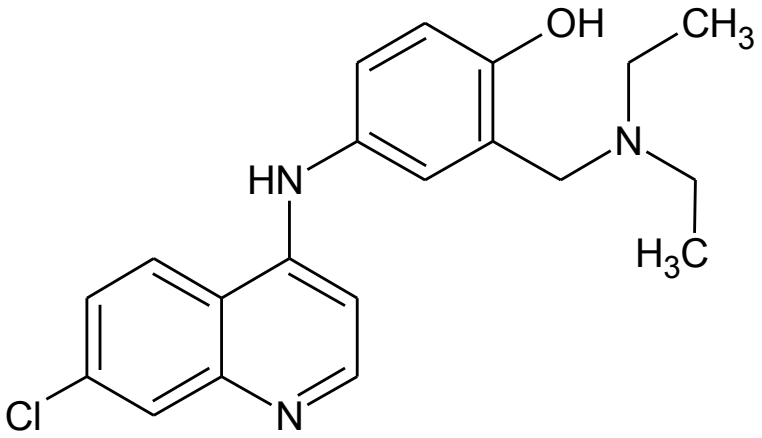
After the Second World War, American scientists created more chloroquine-like analogues and a huge screening campaign was thus launched in an effort to increase the rapidly deteriorating arsenal of antimalarial drugs. A new 4-aminoquinalone was found in this process, named amodiaquine (Kouznetsov & Gomes-Barrio, 2009).

Amodiaquine went into widespread use in 1951, both as a treatment and prophylaxis of malaria. During the 1980's it became apparent that amodiaquine caused some serious side effects, which then led to it being removed from the WHO' essential drug list in the early 1990's. Later it was found that the side-effects were the result of prolonged prophylactic use. Side-effects include immune-mediated granulocytopenia, severe neutropenia and hepatotoxicity, though these effects are quite rare. The WHO returned amodiaquine to the essential drug list in 1996 after the 19th Expert Committee decided that the benefits of the treatment outweighed the potential side-effects. As of yet there has been no evidence of agranulocytosis or hepatitis occurring during normal treatment of malaria, but patients who have impaired hepatic function should be monitored throughout treatment (Olliaro & Mussano, 2009).

After being brought back into use, very few countries restarted the use of amodiaquine. Certain countries that kept amodiaquine in the first line treatment have proven amodiaquine to be a very safe alternative option, as there have been no reports of severe adverse effects when not used for long term prophylaxis. The physical properties of amodiaquine is displayed in table 1.4.

1.11.1. Properties

Table 1.4: The physical properties of amodiaquine (United States Pharmacopeia, 2010)

Chemical composition	Mannich-base 4 Amino-quinoline derivative
Structure	$C_{20}H_{22}ClN_3O$
The chemical structure of amodiaquine	
Chemical name	4-(7-chloro-4-quinolyamino)-2-(diethylaminomethyl) phenol
Formulary weight	464.8g/mol
Description	Yellow crystalline powder
Solubility	• 1 in 22 parts water • 1 in 70 parts ethanol (96%) • Practically insoluble in benzene, chloroform and ether
Solution	• 1% solution in water has a pH of 4.0 to 4.8 • 2% solution in water has a pH of 2.6 - 4.6
Decomposition	Between 150-160 °C
Type of antimalarial	Blood schizontocide

1.11.2. Pharmacokinetics

Amodiaquine taken orally is quickly absorbed through the gastrointestinal tract, with peak plasma concentrations being reached within 30 minutes in healthy volunteers and within two hours in patients with malaria. Amodiaquine is then metabolised in the liver to desethylamodiaquine and three other less active compounds. Desethylamodiaquine is inherently effective against malaria parasites, but three times less than the original amodiaquine form. Desethylamodiaquine has an extremely long half life when compared to the original forms (Churchill *et al.*, 1985).

1.11.3. Side-effects

Amodiaquine shares many of the adverse effects that chloroquine does, ie. pruritus, nausea, vomiting and other gastro intestinal symptoms, but it has become known for the more serious, though rare cases of agranylocytosis, severe neutropenia and hepatitis. Cases of severe side effects are extremely rare. A study that was performed in the United Kingdom proved that fatal incidents happened in only 1 of more than 15 500 travellers. The serious side-effects are thought to arise from an immune response after the parent drug has been broken down (Winstanley *et al.*, 1990).

Amodiaquine is, therefore, contraindicated in persons with any hepatic disorders and persons with known amodiaquine hypersensitivity. Amodiaquine is completely contraindicated for use as chemoprophylaxis (Rosenthal & Katzung, 2004).

1.12. Resistance to quinolines

Chloroquine resistance was first identified after a campaign in which table salt was chloroquinated in an effort to treat entire populations in South America (Foley & Tilley, 1997). With the widespread resistance to chloroquine and other 4-aminoquinolines many studies have tried to identify the mechanism through which *Plasmodium* parasites become resistant. The ways in which a parasite can become resistant is as follows:

- The parasite changes the structure of the drug to be less toxic. The parasite changes the target area.
- The parasite over expresses the targeted site, thus decreasing toxicity of the drug.

- The parasite decreases the influx of the drug or increases the efflux of a drug. Thus, the drug concentration is decreased and the drug has less time to apply the mechanism of action. This theory involves a specific transporting mechanism in one form or another (Van Schalkwyk & Egan, 2006).

Many hypotheses exist, with the following attributed specifically to Chloroquine resistance, including the above mechanisms.

- The parasite decreases the amount of chloroquine that accumulates in the digestive vacuole; this could be achieved by changing the pH of the vacuole. Chloroquine accumulation in the digestive vacuole is thought to be, or at least in part, because of the pH difference between the cytoplasm and the digestive vacuole.
- Another theory states that chloroquine resistant parasites have a lower pH, which reduces the amount of free haem, thus removing a chloroquine target (Van Schalkwyk & Egan, 2006).

1.12.1. Cross resistance between quinolines

Some studies have found that when resistance to one type of quinoline antimalarial arises, often the parasites are cross resistant to other quinoline antimalarials. This has definitely been the case with quinine, mefloquine, chloroquine and in certain cases amodiaquine (Daily, 2006). The specific mechanism by which resistance occurs or spreads is widely debated. Recent studies found genes that are thought to be involved in causing resistance. The genes P*f*CRT and P*f*MDR are both involved with transport within the parasite and have been specifically linked to cross resistance between the quinolines (Nakrumah *et al.*, 2009; WHO, 2009).

Resistance to chloroquine has become a widespread occurrence, with the emergence of strains specifically found to be resistant to amodiaquine and other quinolines more worrying (Foley & Tilley, 1997). Strains resistant to amodiaquine and chloroquine have been identified in Cambodia and Thailand (Winstanley *et al.*, 1990). Luckily resistance to both chloroquine and amodiaquine is rare, because amodiaquine resistance has been connected to a decrease in the initial uptake, rather than increased efflux of the drug, which is thought to be the mechanism of action in most cases of chloroquine resistance (Foley & Tilley, 1998). Therefore, amodiaquine was found to be effective for the treatment of malaria even in places where chloroquine resistance occurs (Hawley *et al.*, 1996; Winstanley *et al.*, 1990).

1.12.2. Overcoming resistance

A few strategies may be employed to try and overcome existing resistance and to stop, or at the very least, slow resistance to current antimalarials. The strategies include combination therapies (using two antimalarials with different mechanism of action, which work in synergy), combining antimalarials with drugs that inhibit mechanisms of resistance (also known as "chemosensitisers") or employing drug delivery systems.

1.12.2.1. Combination therapies

Currently this is the main strategy that the World Health Organisation (WHO) prescribes for curbing and controlling antimalarial resistance. The combination prescribed for most areas are artemisinin-based combinations. Each geographic area needs to do regular efficacy studies to make sure that resistance does not occur, or in cases where it does occur, therapies must be changed (WHO, 2009).

1.12.2.2. Chemosensitisers

During studies on antitumor drugs, it was found that verapamil, a Ca^{2+}-channel blocker was able to interfere in the efflux of the drugs from the tumor cells (Tsuruo *et al.*, 1981). This strategy was then tested on *P. falciparum* using a wide variety of chemosensitisers and found to be effective at partially reversing resistance. When employing a strategy such as this, the effect of the added drug, safe dosages, toxicity, as well as the financial implications it proposes, need to be taken into account. Some of the chemosensitisers that have been identified include verapamil, amitriptyline, citalopram, desipramine, nomifenasine and oxaprotaline, just to name a few. Unfortunately in some cases the chemosensitisers need to be given in very high dosages to reverse antimalarial resistance (Ward & Bray, 2001). Van Schalkwyk *et al*. (2001), proposed that to overcome this, the chemosensitisers can be given in non-toxic doses, but in combinations, as the effect of each drug seems to be additive.

1.12.2.3. Drug delivery systems

Drug delivery systems are often an alternative to using other drugs or chemicals to either increase the uptake, reduce resistance or decrease toxicity. Many of the added chemicals or drugs have effects and side-effects of their own. Thus, to be an effective drug delivery system,

the system must increase the effectiveness of the drug without adding unwanted effects or toxicity. Many drugs currently on the market have limited use, because of poor bioavailability, pharmacokinetics or toxicity. These problems can be overcome when an effective drug delivery system is used. The use of liposomes specifically has the following advantages:

- Improved biodistribution and pharmacokinetics.
- Decrease in toxicity (Often lower doses are required for the same effect).
- Activity against intracellular organisms is increased.
- Activity against extracellular organisms is increased.
- Liposomes can be made to be target specific cells (Drulis-Kawa & Dorotkiewicz-Jach, 2010; Sharma & Sharma, 1997).

1.13. Conclusion

Malaria is a parasitic disease caused by parasites in the *Plasmodium* family. Nearly 500 million cases of malaria are reported each year with a mortality rate of 1 million people each year. Even countries in which malaria has been under control for a few years still have sporadic outbreaks which take many lives in the process; lives which could have been saved. This parasite has a complex lifecycle with two distinct phases, the first of which take place in *Anopheles* mosquitoes and the second phase which occurs in a vertebrate host. This phase in the vertebrate can be split into two phases, the exoerythrocytic and endoerythrocytic phases. Antimalarials are often classified according to the phase where their major antimalarial effect occurs within the parasite lifecycle. Most of the clinical symptoms due to the endoerythrocytic phase. Symptoms of malaria include; fever, headache, chills, body pain and fatigue. Malaria can easily become complicated, which can then become fatal in a very short time.

Resistance has become a huge concern in the past few years, forcing many revisions to treatment guidelines. The *4-aminoquinolines* have been the mainstay of antimalarial treatment for a long time, with chloroquine as the mainstay drug for many years, but wide spread resistance is limiting the usefulness of this drug. Amodiaquine has come into the spotlight as a possible replacement, but the toxicity of this drug is also limiting its usefulness. Strategies to overcome resistance need to be researched to extend the lifetime of treatments currently in use, as the development of new antimalarials is a very expensive process that takes years to complete. The strategies to overcome resistance include, combining therapies with different mechanisms of action, combinations with chemosensitisers as well as the use of effective drug delivery systems.