Chlorproguanil - Dapsone a new drug in the fight against malaria

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SUMMARY

This article is a review of information on a new short acting anti-folate developed for use in treatment for non severe malaria. The information was drawn mainly from published data. This drug was developed at the time chloroquine was failing and a search for a cheap effective alternative was on. Anti folates became the drug of choice to many countries. Sulfadoxine/ pyrimethamine was the anti-folate of choice, but they had a disadvantage of having long half lives that have a high selection pressure for resistance strains. Chloroproguanil (CPG) with dapsone (DDS) are both rapidly eliminated and this is likely to prolong its effective lifetime in the treatment of malaria. Studies done in various countries in Africa have shown that CPG with DDS are effective, have rapid elimination and the resistance require more point mutation than Sulfadoxine/ pyrimethamine. Though the toxicity data is awaited from the field we can deduce from the information available that it is a safe drug and the only adverse event directly attributed to it is aneamia and this occurred in less than 1% of those studied.

Introduction

This is a review of published data in peer reviewed journals and unpublished data from GlaxoSmithKline (GSK) of a new rapidly eliminated anti folates, chloroproguanil (CPG) with dapsone (DDS). It has been estimated by WHO that there are about 300 to 500 million clinical cases of malaria and over one million deaths due to malaria each year [1]. Most of the clinical cases and 90% of the deaths are estimated to occur in sub Saharan African children [2, 3, 4]. There has been an increasing resistance to drugs commonly used in the management of malaria [3,5,6]. Chloroquine and sulfadoxine – pyrimethamine are the most widely available and cheap drugs for malaria in Africa. Treatment failure after choroquine is now ubiquitous in sub-Saharan Africa, and occurs in more than 25% of treated cases throughout East Africa [7,8,9]. The slow elimination of sulfadoxine – pyrimethamine has led to the rapid selection of resistant parasites in many areas of the world, such as Thailand where significant resistance developed in about a decade [6,8,9]. Thus the

need for effective, safe, practicable, and affordable drugs that have lower selection pressure for resistance. Initial work on a new anti-malarial treatment, (chloroproguanil (CPG) with dapsone (DDS) which is marketed as Lapdap was carried out in Kenya (At the Kenya Medical Research Institute) in collaboration with Liverpool University in the 1980s to 1998 when a Public-private partnership was formed between GlaxoSmithKline (GSK), WHO/UNDP/World Bank, Special Programme in Research and Training in Tropical Diseases (WHO-TDR), the UK Department for International Development (DFID) to continue the development of *Lapdap*.

This was after their first in vitro and in vivo experiments had indicated that this new treatment might have advantages over sulfadoxine-pyrimethamine [10,11,12]. Rapid elimination of both CPG and DDS than sulfadoxine-pyrimethamine is likely to prolong the effective lifetime of *Lapdap* as a

treatment for *P. falciparum* malaria. *Lapdap* has also been found to be effective against sulfadoxine-yrimethamine- resistant parasites in Africa. High-level resistance to sulfadoxine-pyrimethamine requires the presence of three or four separate mutations in the gene for dihydrofolate reductase (DHFR) [8,13,14]. Strains of *P. falciparum* in Africa are increasingly found with three mutations, reflecting growing resistance to sulfadoxine-pyrimethamine [13,14,15]. These triple mutants retain sensitivity to *Lapdap* [13], and a fourth mutation is necessary before significant resistance to *Lapdap* occurs [8]. Therefore it is predicted that *Lapdap* will retain efficacy in Africa in the presence of sulfadoxine- pyrimethamine resistance.

The Pharmacology and Stracture of CPG and DDS

CPG [N-(3,4-diclorophenyl)-N1-(1-methylethyl)imidodicarbonimidic diamidel], also known as chlorproguanil , has the chemical formula $C_{11}H_{16} C_{13}N_5$ (see stracture below)
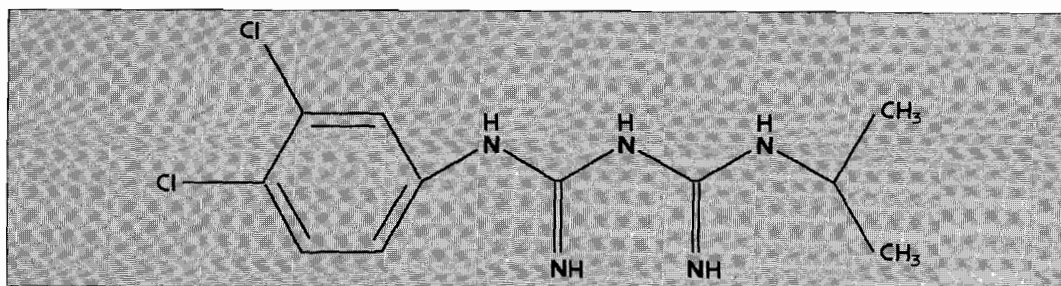
DDS[4,4'-diaminodiphenyl silphone], als know as dapsone, has the chemical formula $C_{12}H_{12}N_2O_2S$ (see stracture below)
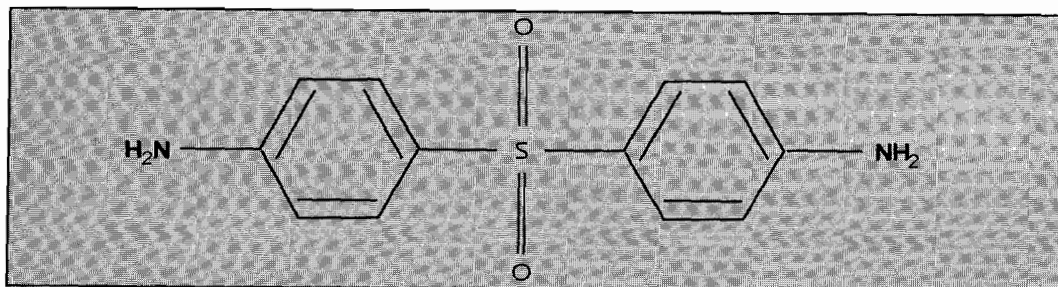
Chloroproguanil (CPG) with dapsone (DDS) belongs to a class of antimalarials known as antifolates. This class of drugs works by blocking the synthesis of tetrahydrofolate, which is essential for DNA synthesis in the asexual replication (schizogony) of the blood stages of the malaria parasites[16]. The components of *lapdap* act synergistically on different parts of folate metabolism in *Plasmodium* parasites. The two components of *Lapdap,* CPG and DDS act sequentially to block two of the key enzymes involved in the synthesis of tetrahydrofolate[16]. CPG inhibits the step mediated by dihydrofolate reductase (DHFR), while DDS inhibits the step mediated by dihydropteroate synthase (DHPS) [16]. CPG is metabolized to chlorcycloguanil (CCG) which inhibits DHFR. Inhibiting this enzyme blocks the formation of tetrahydrofolic acid, an essential co-factor in nucleic acid synthase, preventing the asexual reproduction of the parasites [17]. DDS is

thought to act as a competitive inhibitor of DHPS in malaria species, blocking the conversion of para-aminobenzoic acid (PABA) to hydrofolic acid [18]. This results in inhibition of folate metabolism and subsequently blocking of DNA synthesis.

Chlorproguanil (CPG) is closely related to the antimalarial proguanil (PG). Studies of PG have shown that the parent compound is broken down by hepatic cytochrome P450 isoenzymes to form two main metabolites: a biguanide (N^1-p-chlorphenylguanide) and a triazine, cycloguanil (1-p-chlorphenyl-2), 4-diamino-6-6-dimethyl-1, 6-dihydro-1, 3-triazine) [19]. The triazine metabolite of PG, cycloguanil, is significantly more potent against *P. falciparum* than the parent compound, and it is believed that CPG behaves in a similar fashion – its major metabolite, chlorcycloguanil (CCG), providing the antimalarial effects of the drug [19].

Dapsone (DDS) is 50-90% bound to plasma proteins. In the liver, it is acetylated to monoacetyl and diacetyl derivatives, the major metabolite being monoacetyldapsone (MADDS) [20]. Hydroxylation also takes place in the liver, where DDS and MADDS are converted to DDS-monohydroxylamine and MADDS-hydroxylamine, respectively. These hydroxylated compounds are further partly conjugated as N-glucoronides and N-sulphates, which are excreted in the urine. [20]

Pharmacokinetics

Both components of *Lapdap* are absorbed following oral administration, with median peak concentrations of CPG and DDS being achieved within 4 hours, while those for the two metabolites CCG and MADDS being achieved after 8 and 2.5 hours respectively. The corresponding median C_{max} values were 120ng/ml (CPG), 25ng/ml (CCG), 1,900ng/ml (DDS) and 380 ng/ml (MADDS). Elimination half-lives for the two parents and their metabolites are similar, at around 32 h, 36 h, 29 h and 29 h, respectively for CPG, CCG, DDS and MADDS [17,18]. A study to measure pharmacokinetic parameters for each of the compounds and their major metabolites

when the drugs were given alone and together in healthy adults (n=23) showed that CPG and DDS administered together did not affect each other's rate of absorption, or the rate of appearance of the metabolites in plasma. Giving the two drugs together do not affect the C_{max}, $t_{1/2}$, AUC_{0-00} or AUC_{0-tn} of CPG, DDS or MADDS. However for CCG, C_{max}, AUC_{0-00} and AUC_{0-tn} were lower by approximately 35%, 25% and 30% respectively, when the two drugs were administered together, compared with CPG given alone [17]. The study concluded that there was no major pharmacokinetic interaction between CPG and DDS and that giving the drugs together resulted in similar plasma concentrations to those when the drugs were given separately. However, plasma concentrations of CCG were slightly lower when the two drugs were administered together than when CPG was given alone [17].

Special populations

Investigation to find out the differences in the pharmacokinetics of *Lapdap* between malaria patients and healthy volunteers, and between children and adults were conducted. Pharmacokinetic data were collected prospectively from three different groups of subjects: healthy volunteers, adults suffering from *P. falciparum* malaria in Zambia, and children suffering from *P. falciparum* malaria in Gambia [21].

For CPG, no differences were observed between the healthy volunteers and the patients suffering from malaria. Children had significantly lower values for the absorption rate constant (k_a) for CPG, and clearance (CL/F) was significantly correlated with body weight [21]. For DDS, no pharmacokinetic differences were observed between the healthy volunteers and the patients suffering from malaria. CL/F and volume of distribution (V/F) were significantly positively correlated with body weight. Children were observed to have higher values for CL/F and V/F, independent of body weight, although the difference did not reach statistical significance for either parameter [21]. The results confirm that the dose recommendation for *Lapdap*

should be adjusted for body weight and show that malaria infection does not affect the pharmacokinetic parameters for CPG or DDS [21]. There have been no studies to investigate the pharmacokinetics of *Lapdap* in elderly patients or patients with renal or hepatic impairment.

The development of resistance

As with Lapdap, in sulfadoxine-pyrimethamine, an inhibitor of DHFR (phyrimethamine) works synergistically with the inhibitor of DHPS (sulfadoxine). However, in contrast to Lapdap, sulfadoxine-pyrimethamine is eliminated slowly (half-lives of suldoxine, 116 hours and pyrimethamine, 81 hours) [22]. This property is potentially useful, as it provides a period of chemoprophylaxis after treatment but, as concentrations of the two drugs decline, a 'resistance selection window' may be opened [3]. It is useful to consider three time periods after dosing with a long-acting antimalarial like sulfadoxine-pyrimethamine:

1. **Between dosing and day 14:** synergistic concentrations of pyrimethamine and sulfadoxine are able to eliminate most strains of *P. falciparum*

2. **Between days 15 and 51:** This period is the 'resistance selection window', where concentrations of the two drugs are enough to kill sensitive strains, but, if the patient becomes re-infected with a mixed population that includes both sensitive and resistant strains, the resistant sub-population will survive, while the sensitive one is killed. The importance of this event is that the individual may go on to become ill again and the resistant strain may be passed on to a new host.

3. **From day 52 onwards:** concentrations are no longer high enough to cause any parasite suppression.

Chlorproguanil-dapsone treatment with a very short half life helps to close the 'resistance

selection window' [3]. Chlorproguanil-dapsone has been shown to be effective as a re-treatment drug after failure of pyrimethamine-sulfadoxine. In one of the studies 28 (61%) of 46 children retreated with pyrimethamine-dapsone were still parasitaemic at day 7, compared with three (7%) of 44 children retreated with chloroproguanil-dapsone. Resistance to pyrimethamine-sulfadoxine increased from 45% (156/348) at the first treatment to 61% (28/46) after retreatment. 83 of 85 parasite isolates collected after the first pyrimethamine-suldoxine treatment, and before and after the second treatments with pyrimethamine-sulfadoxine and chlorproguanil-dapsone showed triple-mutant DHFR alleles, associated with a variety of DHPS mutations [13, 23].

Clinical safety profile

The common adverse events in both the *Lapdap* and sulfadoxine-pyrimethamine groups [24], is laboratory abnormalities, and for many patients, these could be related to pre-existing conditions, such as other parasitic infections. The clinical symptoms reported are often difficult to distinguish from symptoms associated with malaria infection, and it is notable that the frequency of adverse events is similar for both *Lapdap* and sulfadoxine-pyrimethamine treatment [24]. 10% of patients in the principal clinical study experienced adverse events that were thought to be related to the study medication. For *Lapdap* the most common treatment-related adverse events were red blood cell disorders (5%) and gastrointestinal (GI) disorders (2%). All other treatment-related adverse events had an incidence of less than 1% [24].

Haematological and cardiovascular adverse effects

Lapdap is well tolerated. There are rare reports of clinically relevant haematological adverse events. Malaria infection itself produces anaemia, which treatment may worsen, and one component of Lapdap, dapsone (DDS), has been known to produce methaemoglobinaemia (medHB) at therapeutic

doses. There are also reports of haemolysis after treatment with DDS, due to glucose 6 phosphate dehydrogenase (G6PD) deficiency, which is relatively common in African populations (around 20%) [25]

In the principal safety and efficacy clinical study, anaemia was reported in 13% of patients in both the sulfadoxine-pyrimethamine and Lapdap groups [25]. In both the sulfadoxine-pyrimethamine group and the Lapdap group, mean haemoglobin increased. At visit 5, there was a significant difference between treatment groups (P<0.0001) but, at visit 6, the difference was no longer significant (P=0.7121). It was shown in that, for the population studied, there was a greater negative effect on haemoglobin after Lapdap treatment than after treatment with sulfadoxine-pyrimethamine, but this disappeared by the second week after treatment. The reasons for this difference are probably complex, but may include potential detrimental factors such as MetHb and G6PD deficiency.

Methaemoglobinaemia (MetHb) is difficult to assess, and this was only possible at one of the first treatment sites in the principal Lapdap clinical trial (Kilifi, Kenya) [25]. Results from that location, in children with malaria, showed that only patients treated with Lapdap experienced increases in MetHb during treatment. Statistically significant rises (>10% above normal) were seen in 7% of the Lapdap-treated patients, but none of these patients experienced clinical symptoms or cyanosis (these usually occur at about 20% above normal values). These findings were are as expected, and it was concluded that increases in MetHb caused by 3 days' exposure to DDS during Lapdap treatment are limited and unlikely to be of clinical importance [24,26]. Haemolysis caused by G6PD deficiency was studied in the principal Lapdap safety and efficacy study (n=1,850) [24] analysis of the data showed that there was a ten-fold greater likelihood of a patient with G6PD deficiency experiencing a fall in haemoglobin >2 g/dl if they were treated with Lapdap, compared with sulfadoxine-

pyrimethamine. However, for a fall in haemoglobin >4 g/dl, there was no statistically significant difference in treatment used [24]. Haemolytic anaemia was an uncommon reported event, seen in only five patients, of whom just three were found to have G6PD deficiency [26].

Lapdap is as well tolerated as sulfadoxine-pyrimethamine. Haematological adverse events were: reversible; not unexpected; generally not clinically significant. G6PD testing is not required before prescribing Lapdap.

Cardiovascular assessments were carried out in healthy adults within the two clinical pharmacology studies in the Lapdap clinical development programme [17,27]. Measurements included blood pressure and pulse rate (conducted regularly throughout the studies) and electrocardiogram (ECG) assessments. In total, 60 subjects were repeatedly evaluated. The studies found that, with Lapdap treatment, vital signs were essentially unchanged from baseline and there were no significant shifts in any of the ECG assessments [17]. In a rising dose-tolerance study, there was no evidence of any vital sign changes associated with increasing doses of chlorproguanil (CPG) [25,26]. In the principal Lapdap pharmacological study, no important changes in ECG parameters were observed with Lapdap treatment – in particular treatment did not lead to prolongation of the QT interval [17].

Serious adverse events

In all the sited studies, both the Lapdap group and the sulfadoxine-pyrimethamine group, serious adverse events are reported with a similar frequency. Over all the studies in the Lapdap clinical development programme [17,24,27,28,29,30,31] thirty-five of two thousand and eighty one Lapdap patients (1.7%) and fifteen of the eight hundred and seventeen sulfadoxine-pyrimethamine patients (1.8%) experienced serious adverse events [25]. Patients were half as likely to require hospitalization on taking Lapdap compared with sulfadoxine-pyrimethamine (not

statistically significant). Severe malaria resulting in hospitalisation was the commonest event, with 11 *Lapdap* patients (0.5%) and ten sulfadoxine-pyrimethamine patients (1.2%) affected [26]. Anaemia, including haemolytic anaemia, is the second most common serious adverse event, which affected 14 of the 2000 *Lapdap* patients (0.7%) and two sulfadoxine-pyrimethamine patients (0.2%) [26].

In *Lapdap* studies, five *Lapdap* patients and two sulfadoxine-pyrimethamine patients experienced various central nervous system symptoms, including convulsions and encephalopathy. All these symptoms occurred early in treatment, and this cannot be difrentiated from natural history of malaria when progression to cerebral malaria is most likely [26]. Other severe adverse events, including fever, thrombocytopaenia and vomiting, are all common to acute malaria. The withdrawals from treatment reflected the overall pattern of serious adverse events [26]. Four *Lapdap* patients (0.3%) and two sulfadoxine-pyrimethamine patients (0.5%) were withdrawn due to severe anaemia. Malaria requiring inpatient treatment caused withdrawal of seven *Lapdap* patients (0.5%) and 11 sulfadoxine-pyrimethamine treated patients (3.0%), and the difference between treatments was attributed to the relatively lower efficacy of sulfadoxine-pyrimethamine at some of the sites. In the supporting studies, two children were withdrawn for severe vomiting, and one had severe pruritus without rash [26]. Overall, the frequency of withdrawal was low and, apart from withdrawals due to haemolytic anaemia in G6PD-deficient patients, it was not possible to assess to what extent these withdrawals were due to medication [26]. In malaria infection, many of the symptoms reported (anaemia, vomiting, rash) are seen regardless of medication is used. The only death reported across all the studies in the clinical development programme was in a 10-month-old boy, with low weight, who was successfully treated twice with *Lapdap* over a 2-month period. He later returned to hospital with a cough and fever and died 36 hours after being discharged the next day. No firm cause

of death was identified, although it was most probably due to a lower respiratory tract infection. However, malaria cannot be ruled out as no blood slide was carried out. This death occurred 2 months after the most recent *Lapdap* treatment [29].

Precautions in administration of Chlorproguanil and Dapsone
Patients with the history of G6PD deficiency Methaemoglobin reductase deficiency Haemoglobin M or E or Porphyria are more susceptible to the haemolytic effects associated with dapsone. Caution is required when administering *Lapdap* to these patients. Due to the risk of haemolytic anaemia which is associated with dapsone, caution is required when treating patients with a history of intravascular haemolysis, pre-existing severe anaemia, or cardiac or pulmonary disease. mg Tablets should be discontinued immediately, if signs of haemolysis (anaemia, dark coloured urine) or methaemoglobinaemia (cyanosis) develop during administration. Alternative antimalarial treatment should be commenced without delay, in the event that a patient deteriorates whilst taking Lapdap. The safety and efficacy of Lapdap for the treatment of malaria has not been evaluated in pediatric patients who are less than 3 months of age.

Recommended Dosage
The recommended dose is 2mg/kg for chroloproquanil and 2.5mg/kg for Dapsone. Lapdap is available in two tablet strengths for adult and paediatrics: 15/18.75 mg per tablet, and 80/100 mg per tablet respectively.

Discussion
Resistance to chloroquine is now so widespread, that in some areas, it is not considered to have any clinical efficacy [6]. Similarly, pyrimethamine is no longer used alone because of widespread resistance, and resistance to sulfadoxine-pyrimethamine is increasingly reported.[7,8,32]. The further spread of resistance to sulfadoxine-pyrimethamine will have an enormous effect on the management of malaria in Africa, since there are currently no affordable alternative

treatments. In South East Asia, most strains of malaria became resistant to sulfadoxine-pyrimethamine within a few years of its widespread use, but in sub-Saharan Africa it has, until recently, remained relatively effective [6].

Chlorproguanil and Dapsone is an effective drug in the treatment of non severe malaria even in a population in which there is resistant to pyrimethamine/sulfadoxine. Significant resistance to Chlorproguanil and Dapsone in the population will most likely take a much longer period as they both have short half life and the resistance require at least four point mutation. It is a safe drug and the only serious adverse that can directly be attributed to this drug is anemia and occurs in less than 1% of the study patients.

The development of this drug that has recently been launched in several African countries is a welcome development in a situation where we are running out of cheap option for effective treatment of malaria.

References

1. World Health Organization. The World Health Report 2000; Health systems: improving performance. World Health Organization. Geneva. 2000.
2. World Health Organization. Economic costs of malaria are many times higher than previously estimated (press release). WHO/28. World Health Organization. Geneva, Switzerland. 2000
3. Bloland PB; Ettling M and Meek S. Combination therapy for malaria in Africa: hype or hope? *Bulletin of the World Health Organization 2000*; **78**:1378-1388.
4. Bloland PB. Drug resistance in malaria World Health Organization, Publication WHO/CDS/CSR/DRS/2001, Geneva. 2001.
5. Winstanley PA. Chemotherapy for falciparum malaria: the armoury, the problems and the prospects. *Parasitology Toda.y* 2000; **16**:146-153.
6. Gottlieb S. New strain of malaria reported in Africa. *British Medical Journal.* 2001; **323**:251.
7. Deloron P; Mayombo J; Le Cardinal A; J. Mezui-me – Ndong; C. Bruzi-Baert; F. Lekoulou and N.Elissa. Sulfadoxine-pyrimethamine for the treatment of *Plasmodium falciparum* malaria in Gabonese children. *Transcations of the Royal Society of Tropical Medicine and Hygiene Hygiene. 2000*; **94**:188-190.
8. Watkins WM; Mberu EK; Winstanley PA; and Plowe CV. The efficacy of antifolate combinations in pharmacodynamic and pharmacokinetic analyses. *Parasitology Today.* 1997; **13**:459-464.
9. Winstanley PA; Watkins WM; Muhia D; Simon Szwandt; Evans Amukoye and Kevin Marsh. Chloroproguanil-dapsone for uncomplicated falciparum malaria in young children: pharmacokinetics and therapeutic range. *Transactions of the Royal Society of Tropical Medicine and Hygiene.* 1997; **91**:322-327.
10. Watkins WM; Brandling-Bennett AD; Nevill CG; Carter JY; Boriga DA; Howells RE and Koech DK. Chlorproguanil/dapsone for the treatment of non-severe Plasmodium falciparum malaria in Kenya: a pilot study. *Transactions of the Royal society of Tropical Medicine and Hygiene* 1988; **82**:398-4-3.
11. Winstanley PA; Mberu EK; Szwandt IS; Breckenridge AM and WatkinsWM. *In vitro* activities of novel antifolate drug combinations against *Plasmodium falciparum* and human granulocyte CFUs. *Antimicrobial Agents and Chemotherapy* 1995; **39**:948-952.
12. Amukoye E; Winstanley PA; Watkins WM; Snow RW; Hatcher J; Mosobo M; Ngumbao E; Lowe B; Ton M; Minyiri G and Marsh K. Chlorproguanil-dapsone: effective treatment for uncomplicated falciparum malaria. *Antimicrobial Agents and Chemotherapy.* 1997; **41**:2261-2264.
13. Mutabingwa T ; Nzila A ; Mberu EK ; Nduati E ; Winstanley PA ; Hills E and Watkins WM. Chlorproguanil-papsone for treatment of drug-resistant falciparum malaria in Tanzania. *Lancet* 2001;**358**:1218-1223.
14. Nzila-Mounda A; Mberu EK; Sibley CH; Plowe CV; Winstanley PA and Watkins WM. Kenyan *Plasmodium falciparum* field

isolates: correlation between pyrimethamine and chlorcycloguanil activity in vitro and point mutations in the dihydrofolate reductase domain. *Antimicrobial Agents and Chemotherapy.* 1988; **42**:164-169.

15. Khan B; Omar S; Kanyara JN; Warren-Perry M; Nyalwidhe J; Peterson DS; Wellems T; Kaniaru S; Gitonga J; Mulaa FJ and Koech DK. Antifolate drug resistance and point mutations in *Plasmodium falciparum* in Kenya. *Transactions of the Royal Society of Tropical Medicine and Hygiene.* 1997; **91**:456-460.

16. Curtis J. Chloroproguanil-dapsone. The University of Liverpool. *Current Opinion in Anti-infective Investigational Drugs 2000*; **2**: 99-102.

17. GlaxoSmithKline. Data on file (RSD-1101LL3/1).2001.

18. Van Dyke K. DDS: mechanisms of action and structure-activity relationships. *Archives Internationales de Pharmacodynamie et de Therapie.* 1972; **200**:147-152.

19. Robertson GI. Experiments with antimalarial drugs in man. V. Experiments with an active metabolite of proguanil and an active metabolite of 5943. *Transactions of the Royal Society of Tropical Medicine and Hygiene.* 1957; **51**:488-492.

20. Zuidema J, Hilbers-Modderman ESM & Merkus FWHM. Clinical pharmacokinetics of dapsone. *Clinical Pharmacokinetics.* 1986; **11**:299-315.

21. GlaxoSmithKline. Data on file (RSD-101N4N/1). 2001.

22. Winstanley PA; Watkins WM; Newton CR; Nevill C; Mberu EK; Warn PA: Waruiru CM; Mwangi IN; Warrel DA and Marsh K. The disposition of oral and intramuscular pyrimethamine/sulphadoxine in Kenyan children with high parasitaemia but clinically non-severe falciparum malaria. *British Journal of Clinical Pharmacology.* 1992; **33** (2):143-148.

23. Nzila AM; Mberu EK; Sulo J; Winstanley PA; Sibley CH and Watkins WM. Towards an understanding of the mechanism of pyrimmethamine-sulfadoxine resistance in Plasmodium falciparum: genotyping of dihydrofolate reductase and dihydropteroate synthase of Kenyan parasites. *Antimicrobial Agents and Chemotherapy.* 2000; **44**: 991:991

24. GlaxoSmithKline. Data on file (RSD-101LL2/1).2001.

25. Town M; Bautista JM; Mason PJ and Luzzatto L. Both mutations in G6PD A-are necessary to produce the G6PD deficient phenotype. *Human Molecular Genetics.* 1992; **1** (3):171-174.

26. GlaxoSmithKline. Data on file (Lapdap: Clinical expert report). 2001

27. GlaxoSmithKline. Data on file (RSD-101LL4/1). 2001

28. GlaxoSmithKline. Data on file (RSD-101LL5/1). 2001

29. GlaxoSmithKline. Data on file (RSD-101KHX/1).2001

30. GlaxoSmithKline. Data on file (RSD-101KHT/1).2001

31. GlaxoSmithKline. Data onfile (RSD-101LL6/1). 2001

32. White NJ; Nosten F; Looareesuwan S; Watkins WM; Marsh K; Snow RW; Kokwaro G; Ouma J; Hien TT; Molyneux ME; Taylor TE; Newbold CL; Ruebush TK; Danis M; Greenwood BM; Anderson RM and Olliaro P. Averting a malaria disaster. *The Lancet.* 1999; **353**:1965-1967.