A rising-dose tolerance study in healthy human subjects, examining increasing doses of chlorproguanil with a constant dose of dapsone

Dissertation submitted to the University of Cape Town Faculty of Health Sciences for the degree of MMed part 3 in Clinical Pharmacology.

Candidate: Dr Marc Blockman

BPharm, MbChB

Supervisor: Professor P Smith

Principal Specialist Scientist, Department of Clinical Pharmacology, University of Cape Town and Groote Schuur Hospital

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SYNOPSIS

The emergence of pyrimethamine-resistant *Plasmodium falciparum* malaria necessitates the investigation of alternative drug regimens to treat acute, uncomplicated malaria caused by this organism. It has been demonstrated in vitro that chlorcycloguanil (CCG), the active metabolite of chlorproguanil, is effective in killing pyrimethamine-resistant *P.falciparum*, when used in combination with the sulphone-dapsone. In paediatric patients, one hundred per cent clinical cure was achieved after three doses of 1.2mg/kg/day and 2.4mg/kg/day of chlorproguanil and dapsone respectively. Previously, it was not known whether the 1.2mg/kg/day dose of chloproguanil is optimum and no dose-limiting toxicity testing had been performed.

In this study we show in twelve healthy human volunteers that doses of chlorproguanil 1.2mg/kg and 2.0mg/kg in combination with a fixed dose of dapsone (2.4mg/kg), are well tolerated. At higher doses of chlorproguanil (2.8mg/kg and 3.6mg/kg), moderately severe, but predictable, adverse events occurred in most subjects. No new adverse events were observed.

Kinetic data indicates that saturation of the metabolism of chlorproguanil to CCG occurs at 2.0mg/kg. We propose, therefore, that doses above 2.0mg/kg would only serve to increase adverse events without augmentation of the therapeutic activity of chlorproguanil.

This study has been presented as a Poster at the ASTMH 50th annual Meeting in Atlanta, November 11-15th 2001.

ABSTRACT

Title

A rising-dose tolerance study in healthy human subjects, examining increasing doses of chlorproguanil with a constant dose of dapsone.

Objective(s)

1. To define the dose-limiting toxicity of chlorproguanil (CPG), given in combination with a fixed dose of dapsone (DDS), in healthy volunteers.

2. To determine the relationship between the dose of CPG and the area under the curve of its active metabolite, chlorcycloguanil (CCG).

Study Design

This was an open label, rising dose-tolerance study, conducted over an eight-week period, to define the dose-limiting toxicity of CPG given in combination with a fixed dose of DDS in healthy human volunteers. Each dosing interval was one week, with the drugs being taken for the first three days of each dosing week. There was a washout period of one week between dosing intervals. During each dosing week, blood samples were taken for the measurement of circulating drug and metabolite levels, as well as safety data. Subjects were assessed throughout the test week for any adverse drug experiences.

Study Population

Twelve healthy male subjects between 18 years and 60 years of age, who fulfilled the entry criteria, and who had given their written consent.

Treatment and Administration

CPG was supplied by SmithKline Beecham, UK, as 20 mg tablets. The batch number was LOT EH 389A. DDS was supplied as 100 mg tablets by Lennon Ltd., South Africa. The batch number was MM10790.

The study drug was administered in sequence as follows:

(a) CPG 1.2 mg/kg plus DDS 2.4 mg/kg daily for three days

(b) CPG 2.0 mg/kg plus DDS 2.4 mg/kg daily for three days

(c) CPG 2.8 mg/kg plus DDS 2.4 mg/kg daily for three days.

(d) CPG 3.6 mg/kg plus DDS 2.4 mg/kg daily for three days

In addition, there was a washout period of seven days between each Phase.

Evaluation Criteria

Efficacy Parameters

No efficacy end-points were defined in this study.

Safety Parameters

To record subjective adverse events, including nausea, diarrhoea, abdominal pain, headache, fatigue, pruritus and dizziness during each of the four study Phases.

To record objective adverse events, including rash, mouth ulcers, methaemoglobinaemia, fall in plasma haptoglobin concentration and a rise in plasma urea, creatinine, gamma GT, alkaline phosphatase and bilirubin.

Pharmacokinetics

The following pharmacokinetic parameters were derived for CPG, DDS and mono acetyl dapsone (MADDS) for each dose of CPG (Phase 1 to 4) on day 0

AUC_{0-t}	Area under the concentration-time curve over the sampling period
C_{max}	Peak serum concentration from the measured data
t_{max}	Time to peak serum concentration from the measured data
k	Elimination rate constant

On days 1 and 2, the following parameters were derived for CPG, DDS and MADDS for each dose of CPG (Phase 1 to 4): AUC_{0-t}, C_{max} and t_{max}.

The pharmacokinetic parameters derived for CCG, for each dose of CPG (Phase 1 to 4) on days 0, 1 and 2 were: AUC_{0-t}, C_{max} and t_{max}.

RESULTS

Adverse Events

There were four subjects (33%) who experienced one or more events during Phase 1 1.2 mg/kg), nine subjects (75%) during Phase 2 (2.0 mg/kg), 11 subjects (92%) during Phase 3 (2.8 mg/kg) and 12 subjects (100%) during Phase 4 (3.6 mg/kg).

Adverse event	CPG Dose (mg/kg)			
	1.2 (N=12)	2.0 (N=12)	2.8 (N=12)	3.6 (N=12)
Headache	0	0	0	1 (8.3)
Anorexia	0	7 (58.3)	0	1 (8.3)
Abdominal Pain	0	0	0	1 (8.3)
Diarrhoea	0	0	2 (16.7)	1 (8.3)
Nausea	1 (8.3)	5 (41.7)	11 (91.7)	12 (100)
Stomatitis, Ulcerative	3 (25.0)	1 (8.3)	0	0
Tooth Disorder	0	0	0	1 (8.3)
Vomiting	0	2 (16.7)	7 (58.3)	11 (91.7)
Fever	0	0	1 (8.3)	0
Malaise	0	0	0	1 (8.3)

Only one event recorded during the study was considered unlikely to be related to the study drug. This was fever experienced by a subject on day 1 of Phase 3 (2.8 mg/kg). The relationship to study medication for four events was unknown: One volunteer Phase 4 malaise, anorexia, nausea and tooth disorder.

Serious Adverse Events

There were no serious adverse experiences, and no unexpected toxicity was found in any of the study subjects.

Withdrawals Due to Adverse Events

There were no withdrawals from the study.

Laboratory Data

There were dose-related increases in albumin, bilirubin (total) and bilirubin (unconjugated) and dose related decreases in haematocrit and haptoglobin. None of the changes within any individual subject were considered to be clinically significant. No remarkable changes were seen in any of the other laboratory parameters assessed.

Vital Signs

Electrocardiograms were performed prior to inclusion in the study and then on days 0 and 2 of each Phase of the study.

Pharmacokinetics

As expected CPG showed dose-related increases in pharmacokinetic parameters. CCG also showed dose-related increases but the increase was not linear with respect to the parent compound. C_{max} and t_{max} values for CCG are approximate as conversion of CPG to CCG was incomplete at 9 hours in a number of subjects.

For DDS and MADDS, C_{max} values tended to decrease with increasing dose of CPG. Elimination was slow in both cases and incomplete by the end of the 9-hour sampling period.

		Pharmacokinetic Parameter (mean/median value at Day 0)					
	CPG dose mg/kg	AUC_{0-t} ng.h/mL(±SD)		C_{max} ng/mL(±SD)		t_{max} (hours)	k
CPG							
	1.2	710.4	(216.3)	107.5	(33.1)	2.0	0.122
	2.0	1692.2	(368.6)	258.9	(44.9)	4.0	0.111
	2.8	1520.	(295.7)	231.4	(45.3)	4.0	0.094
	3.6	2154.8	(421.8)	330.2	(51.5)	4.0	0.112
CCG							
	1.2	76.3	(41.5)	16.5	(7.2)	9.0	NR
	2.0	137.5	(46.6)	25.7	(8.3)	6.0	NR
	2.8	142.4	(55.1)	28.6	(9.7)	9.0	NR
	3.6	154.8	(55.4)	30.7	(9.8)	9.0	NR
DDS							
	1.2	21962	(6935)	3268	(981)	2.0	0.062
	2.0	23555	(3422)	3313	(575)	2.0	0.051
	2.8	20423	(4214)	3047	(575)	2.0	0.042
	3.6	19724	(5173)	2946	(605)	2.0	0.045
MADDS							
	1.2	5682	(4851)	902	(646)	2.0	0.076
	2.0	5745	(3722)	875	(593)	2.0	0.052
	2.8	4696	(3290)	727	(528)	2.0	0.043
	3.6	4511	(3150)	727	(436)	2.0	0.047

Conclusions

- Doses of CPG above 2.0 mg/kg in combination with 2.4 mg/kg DDS are associated with a marked increase in the incidence of unwanted effects such as nausea and vomiting.

- Increasing CPG dose increases CCG levels, but the effect is not linear.

- The apparent plateau of CCG levels is achieved at a dose of CPG of 2mg/kg without significant additional plasma concentrations being achieved with higher CPG doses. Any marginal benefits are counterbalanced by side effects.

- 2.0 mg/kg CPG is recommended as the dose of choice when used in combination with 2.4 mg/kg DDS.

Introduction

Although falciparum malaria is a problem throughout the tropics, the greatest concentration of disease is found in sub-Saharan Africa: at a conservative estimate the disease kills one million people per year in Africa, mainly young children, and causes crippling loss of earnings in very poor families[9]. For logistic reasons chemotherapy of uncomplicated malaria is currently, and is likely to remain, the principal means of disease "control" in Africa. Chloroquine has been the mainstay of such chemotherapy, but the prevalence rate of clinical chloroquine-resistance in many areas is now such that alternative drugs are needed[10]. Antifolate drugs are considerably cheaper than other alternatives[11,12] including (halofantrine, mefloquine and artemisinin-derivatives) and pyrimethamine-sulfadoxine (SP) is becoming widely used as the "successor" to chloroquine - especially in high-risk patients like young children. However, pyrimethamine and sulfadoxine are both eliminated slowly[13] favouring the selection of resistant parasites[14], and widespread use is likely to result in rapid development of clinical drug-resistance, as happened in South East Asia[9]. This would be disastrous in the absence of affordable alternative drugs.

In contrast the antifolate combination chlorproguanil-dapsone, which has been shown to clear asymptomatic falciparum parasitaemia,[3] is eliminated rapidly from the body[1]. This may impose a smaller "selection pressure for resistance" than pyrimethamine-sulfadoxine. Furthermore, in-vitro data suggest that the combination of chlorcycloguanil, the active metabolite of chlorproguanil plus dapsone has considerably greater activity against a Kenyan pyrimethamine-resistant strain of *Plasmodium falciparum* than pyrimethamine-sulfadoxine, but is no more toxic to human bone marrow cells in culture[2]. In other words, the introduction of chlorproguanil-dapsone

could prolong the useful lifetime of antifolate drugs in Africa; furthermore, chlorproguanil-dapsone shows promise as an affordable replacement for pyrimethamine-sulfadoxine, once resistance to this combination has developed.

The anti-malarial drug chlorproguanil (CPG) was used extensively for chemoprophylaxis until the mid 1980s, when its production was discontinued; its congener, proguanil, continued to be used.[1] In-vitro work has shown than chlorcycloguanil (CCG), the active metabolite of CPG, is effective against pyrimethamine-resistant strains of *Plasmodium falciparum*, when CPG is combined with the sulphone dapsone (DDS).[2] It has been shown that the combination of CPG and DDS in doses of 1.2 mg/kg/d and 2.4 mg/kg/d, respectively, is highly effective in the treatment of uncomplicated *Plasmodium falciparum* malaria in paediatric patients, achieving 100% clinical cure after seven days.[3]

If clinically necessary, minimum fractional inhibitory concentrations of chlorproguanil and dapsone could be prolonged in three ways: giving further doses of the present fixed-ratio combination, formulating the present fixed-ratio combination for sustained release; or increasing drug doses[4,17,20,21] Giving additional doses would probably reduce compliance, while sustained-release formulation would probably prove too expensive for widespread use in Africa. However, the present data suggest that dose-adjustment is a possibility. The reputation for safety enjoyed by chlorproguanil during its use in chemoprophylaxis, suggests that doses of this drug could be increased safely[3,4,26,27] Furthermore, it is chlorcycloguanil clearance, rather than that of dapsone, which terminates synergy[7,16,20,21]. In contrast, dapsone is dangerous in overdose, causes dose-related haemolysis and methaemoglobinaemia, and achieves acceptable unbound

concentrations at the current dose[4,6,7,17,21]. It seems prudent to fix the dapsone component of this combination while investigating higher chlorproguanil doses.

Pyrimethamine and sulfadoxine are eliminated slowly with half-lives of 81 and 116 hours respectively[4,5,7]. The long-half life of both agents was thought to provide chemoprophylaxis, but also favours the selection of pyrimethamine-resistant strains[13,14]. The mechanism of pyrimethamine resistance is by a genetically determined, drug class specific variation in the enzyme dihydrofolate reductase[14,32]. Therefore strains resistant to pyrimethamine should retain sensitivity to other dihydrofolate reductase inhibitors. It is though that the widespread use of the SP combination in sub-Saharan Africa will result in failure of the combination within a few years[14]. Therefore, the search for a cost-effective readily available alternative has resulted in the use of the chlorproguanil/dapsone combination in clinical trials. Its use relies on the retention of sensitivity to other dihydrofolate reductase inhibitors by the parasite resistant to pyrimethamine[2,3,10].

Both chlorcycloguanil and dapsone have shorter half-lives than pyrimethamine and sulfadoxine being 12.6 and 24,5 hours respectively[5,25,26]. Consequently, it is hypothesised that CPG-DDS may be at least as effective as SP at the moment, and that widespread use of CPG-DDS in place of SP will reduce selection pressure for antifol-resistance[1,2,810].

It is proposed that CPG-DDS may have an advantage over SP in the treatment of acute uncomplicated *Plasmodium falciparum* malaria; however, it remains to be decided whether the dose of CPG used is optimum, and dose-limiting toxicity has never been tested. Higher doses of CPG may confer antiparasitic advantage, but tolerability needs to be studied first. Both proguanil and CPG are known to be

safe[4,6,7]. Idiosyncratic adverse reactions are rare, and dose-related reactions are uncommon and seldom life threatening. There is no recorded fatality following overdose.[4, 5, 6, 7] The largest proguanil overdose recorded is 14.5 g, and there were no sequelae. Data regarding overdose of CPG in humans are lacking.

Dose-related adverse effects of DDS include reversible bone marrow suppression, mucositis and gastro-intestinal upset. Haematological adverse reactions related to DDS are thought to be more common in folate-deficient subjects.[4-7] The dose was therefore fixed at 2.4 mg/kg to avoid the effects of increasing DDS.

The aim of this study was to determine the maximum tolerated dose of CPG when administered with a fixed dose of DDS, in order to optimise the therapeutic effect of the drug in combination with DDS. This was achieved using a rising dose design beginning with the previous maximum dose of CPG investigated.

The clinical effect of CPG is largely attributable to its active metabolite, CCG. Whether there is a linear relationship between the dose of CPG and the area under the curve (AUC) of CCG will be addressed during the rising-dose tolerance study.

The choice of a three day dosage regimen resulted from clinical studies showing that the combination of CPG and DDS in doses of 1.2 mg/kg/d and 2.4 mg/kg/d, given daily for three days, respectively, is highly effective in the treatment of uncomplicated *Plasmodium falciparum* malaria in paediatric patients, achieving 100% clinical cure after seven days.[3] This in contrast to a study utilising a single dose of the combination on day one vs. the three day course. The two treatments produced identical initial clinical response at 48 hours and had parasitaemia clearance at day 7, however recrudescence at day 28 was 70% (single dose) vs. 0.9% (three-day course) [3,8].

1 Objectives

1.1 Primary Objective

To define the dose-limiting toxicity of CPG, given in combination with a fixed dose of DDS, in healthy volunteers.

1.2 Secondary Objectives

To determine the relationship between the dose of CPG and the area under the curve of its active metabolite, chlorcycloguanil (CCG).

2 Methodology

2.1 Study Design

This was an open label, rising-dose tolerance study to determine the dose-limiting toxicity of CPG, given in combination with a fixed dose of DDS. The study was conducted in healthy human subjects over an eight-week period, in four phases. Each phase was one week, with the drugs being taken for the first three days of each phase. There was a washout period of one week between dosing phases.

The study drugs were administered as follows on Days 0, 1 and 2 of each treatment phase:

Phase 1

CPG 1.2 mg/kg plus DDS 2.4 mg/kg daily for three days.

Phase 2

CPG 2.0 mg/kg plus DDS 2.4 mg/kg daily for three days.

Phase 3

CPG 2.8 mg/kg plus DDS 2.4 mg/kg daily for three days.

Phase 4

CPG 3.6 mg/kg plus DDS 2.4 mg/kg daily for three days.

Pharmacokinetic parameters and adverse experiences were assessed on Days 0 to 5 inclusive.

There was a washout period of seven days between Phases.

Subjective adverse events including nausea, diarrhoea, abdominal pain, headache, fatigue, pruritis and dizziness were recorded during each of the four Phases. Objective adverse events (rash, mouth ulcers, methaemoglobinaemia, fall in plasma haptoglobin concentration and rise in plasma urea, creatinine, gamma GT, alkaline phosphatase and bilirubin were also recorded. Sequential blood samples for measurement of the plasma concentrations of CPG, CCG and DDS and mono acetyl dapsone (MADDS) during each of the four Phases were drawn.

2.2 Ethics

The study was conducted in accordance with Good Clinical Practices and the Declaration of Helsinki as amended in Somerset West, Republic of South Africa 1996. The University of Cape Town, Faculty of Medicine, Research Ethics Committee approved the protocol and statement of informed consent. Written informed consent[1] was obtained from each subject prior to entry into the study. Case report forms (CRFs) were provided for each subject's data to be recorded.

2.3 Eligibility Criteria

Subjects were eligible for inclusion in this study provided they satisfied the following inclusion/exclusion criteria.

2.3.1 Inclusion Criteria

Subjects were included in the study if the following criteria were satisfied:

- Male subjects, aged 18 - 60 years of age.
- Written or witnessed oral informed consent had been obtained.
- No significant medical history or abnormal findings on physical examination.
- Preliminary laboratory evaluations within the normal ranges, including routine liver function tests, urea, creatinine, electrolytes, haematological parameters, methaemoaglobin.
- Normal ECG.

2.3.2 Exclusion Criteria

Subjects were excluded from the study if any of the following criteria applied:

- A significantly abnormal medical history or abnormal findings on medical examination or abnormal laboratory results.
- A history of previous liver or renal disease, gastro-intestinal disorders or any paroxysmal abnormality, including seizures and cardiac arrhythmias
- Asthma.
- Anyone who had suffered from or had been treated for a significant illness within three months prior to the commencement of the study.
- A history of drug hypersensitivity.
- Excessive regular use or abuse of alcohol, drugs or medication
- The need for regular medication

- Anyone who had participated in the evaluation of an experimental drug with the previous 12 weeks.

- Smokers

2.4 Study Medication and Administration

2.4.1 Study Medication

The study drugs were stored in a temperature regulated, limited access area. An inventory was maintained of all drugs received and administered. Batch numbers of all study medications were recorded.

Table 2 Dosage strength and batch numbers of drugs used in the study

Study Drug	Formulation	Dose Unit	Batch Numbers
Chlorproguanil	Tablet	20 mg	SmithKline Beecham EH389A
Dapsone	Tablet	100 mg	Lennon Ltd. MM10790

2.4.2 Dosage and Administration (CPG rounded off to nearest 10mg and DDS to nearest 25mg)

The study drugs were administered on Day 0, 1 and 2 of each study Phase as follows:

Phase 1 CPG 1.2 mg/kg plus DDS 2.4 mg/kg daily for three days

Phase 2 CPG 2.0 mg/kg plus DDS 2.4 mg/kg daily for three days

Phase 3 CPG 2.8 mg/kg plus DDS 2.4 mg/kg daily for three days

Phase 4 CPG 3.6 mg/kg plus DDS 2.4 mg/kg daily for three days

There was a washout period of seven days between Phases.

2.5 Compliance with Study Medication

The study drug was administered in the presence of the Investigator and ingestion confirmed. This was recorded in the CRF together with the time and date of dosing. Post treatment times were measured from time of ingestion.

2.6 Prior and Concomitant Medication

Details of all concomitant medications taken during the study were recorded in the CRF together with indication, name of drug, total daily dose, date(s) and route of administration.

2.7 Study Procedures

2.7.1 Pre-study Screening and Enrolment

Pre-study screening and enrolment consisted of the following assessments/procedures and all information was recorded in the CRF:

- Medical history
- Medical examination
- Preliminary laboratory evaluations: full blood count (white blood count and differential, platelets and haemoglobin), urea, creatinine, electrolytes, alkaline phosphatase, gamma GT, bilirubin
- ECG

- Informed consent (written/verbal) was obtained and documented from the subject before study specific procedures were undertaken

In addition to the above, it was ascertained that no drug or medication, including over the counter preparations, might have been consumed in the month preceding the study and that no alcohol or caffeine containing fluids might have been taken for 24 hours on the day preceding the study. It was also required that the subjects had to fast overnight before each dose of study medication.

2.7.2 Treatment Phase

Each volunteer took doses of CPG with a fixed dose of DDS for three consecutive days, at fourteen-day intervals until either the study was complete or the subject withdrawn. The dose of CPG was raised at each stage to four different dosage levels.

Each 14-day period followed the same schedule:

Day 0 (After an overnight fast)

- Pre-dose medical examination
- Medical investigations
- First dose of CPG/DDS
- Blood sampling, pre-dose and then at the following intervals after the dose: 25 minutes; 50 minutes; 2 hours; 4 hours; 6 hours; 9hours.
- Electrocardiogram
- Regular assessments by attending physician

- Completion of adverse drug reaction questionnaire
- Volunteer returned home

Day 1 (After an overnight fast)

- Pre-dose medical examination
- Medical investigations
- Second dose of CPG/DDS
- Blood sampling pre-dose and then at the following intervals after the dose: 4hours; 9 hours.
- Electrocardiogram
- Regular assessments by the attending physician
- Completion of adverse drug reaction questionnaire
- Volunteer returned home

Day 2 (After an overnight fast)

- Pre-dose medical examination
- Medical investigations
- Third dose of CPG/DDS
- Blood sampling pre-dose and then at the following intervals after the dose: 4 hours; 9 hours

- Electrocardiogram
- Regular assessment by the attending physician
- Completion of adverse drug reaction questionnaire
- Volunteer returned home

Day 3

- Blood sampling for drug assay – one sample
- Routine blood investigations
- Medical examination by physician
- Completion of final adverse reaction questionnaire for that level
- Volunteer returned home

2.7.3 Post-treatment Phase (Days 4 and 5)

Study volunteers attended the study centre each morning on Days 4 and 5 for blood sampling for drug assays (one sample was taken on each occasion).

From Day 8 to Day 13 there was a washout period before the next dosage level.

2.7.4 Reasons for Concluding Study

Volunteers could be withdrawn at any time from the study for one or more of the following reasons:

- Withdrawal of consent by the volunteer.
- Any significant adverse event(s) developing at any time during or following study drug administration, serious enough to prevent further dosing.
- Development of a rash which, in the opinion of the attending physician had a moderate or high likelihood of being drug related.
- The need for intercurrent medication, for whatever reason.
- A rise in methaemoglobin to 15% of the total haemoglobin or greater.
- A significant rise in plasma urea, creatinine, bilirubin, alkaline phosphatase or transaminases above the accepted normal ranges.
- A fall in haemoglobin of 2g/dl or more from the pre-study value for that subject.

2.8 Efficacy Assessments

There were no efficacy parameters in this study.

2.9 Safety Assessments

2.9.1 Adverse Experiences

Adverse experiences (AEs) were elicited by the investigator by asking the subject a non-leading question such as ***'Do you feel differently in any way since starting the new treatment?'*** If the subject responded ***'Yes'***, details of the treatment emergent AE and its severity including any change in study drug administration, investigator attribution to study drug, any corrective therapy given and outcome status were

documented on the case report form. Attribution or relationship to study drug was judged by the investigator to be not related, unlikely, suspected (reasonable possibility) or probable. All adverse experiences were coded from the verbatim term according to the WHO Adverse Reaction Terminology (ART) dictionary by body system and preferred term.

Physical examinations were required at the screening visit and on days 0,1,2 and 3 of each Phase of the study. Any adverse changes in the physical examination were to be recorded in the adverse experience pages of the case report forms (CRF).

Clinical laboratory tests were performed at the screening visit and again on days 0-7 of each Phase of the study. These included full blood count, blood biochemistry (urea, creatinine, electrolytes, alkaline phosphatase, gamma GT and bilirubin), and methaemoglobin. In addition, a pre-dose baseline venous blood sample (20 ml) was also taken on days 0 – 2 of each Phase for routine liver, renal, haematological and methaemoglobin assessment. Any abnormalities considered clinically significant were to be recorded in the adverse experience pages of the CRF. In addition, laboratory values of clinical concern were identified and tabulated.

2.9.2 Concomitant Diseases and Drug Events

The number of subjects who experienced at least one concomitant disease or drug event was summarised using frequency counts for each dose of CPG. The incidence of all events was tabulated according to the WHO body system and preferred term by dose of CPG using frequency counts. The number of episodes of each event reported, the number of subjects reporting each event and the percentage of subjects was presented.

The number of subjects suffering from one or more concomitant disease or drug event considered to be related to the study medication (possible, probable/likely, certain and unassessable/unclassifiable) was tabulated by dose of CPG. The incidence of all related events was tabulated according to the WHO body system and preferred term by dose of CPG using frequency counts for each relationship. The number of episodes of each event reported, the number of subjects reporting each event and the percentage of subjects was presented.

The number of subjects reporting concomitant diseases or drug events that the investigator reported to be severe/serious were summarised using frequency counts for each dose of CPG. The incidence of all severe/serious events was tabulated as for related events.

2.9.3 ECG

ECG data manually measured included (ventricular rate, PR interval, QRS duration, QT and QTc) were summarised at each measurement (days 0, 1 and 2) by dose of CPG. Summary statistics (number of non-missing values, mean, standard deviation, median, minimum and maximum) were presented for each parameter.

The change from day 0 to each subsequent measure within a Phase was calculated for each subject and the resulting data summarised by dose of CPG using the same summary statistics as for the absolute values.

2.9.4 Vital Signs and Weight

Vital signs (systolic blood pressure, diastolic blood pressure, heart rate and temperature) were summarised at each measurement (days 0, 1, 2 and 3) by dose of CPG.

Weight was summarised for day 0 by dose of CPG.

2.9.5 Medical history during the study

The number of subjects with normal/abnormal medical history results was summarised at each assessment (days 0, 1, 2 and 3) by dose of CPG for each of the body systems examined.

2.9.6 Physical Examination

The number of subjects with normal/abnormal physical examination results was summarised at each assessment (days 0, 1, 2 and 3) by dose of CPG for each of the body systems examined.

2.10 Pharmacokinetic Assessments

2.10.1 Sampling times

On Day 0 of each study Phase, samples for pharmacokinetic analysis were taken at the following time-points:

Immediately pre-dose and then at 25 minutes, 50 minutes, 2 hours, 4 hours, 6 hours and 9 hours post-dose.

On Days 1 and 2 samples were taken immediately pre-dose and then at 4 and 9 hours post-dose.

On Days 3 to 5 inclusive a single sample was taken in the morning, at approximately 8am.

2.10.2 Specimen preparation

Venous blood samples (10 ml) were collected at each Phase of the study for high performance liquid chromatography (HPLC) assay of CPG, CCG, DDS and mono acetyl dapsone (MADDS). The samples were collected by venepuncture with disposable needles into glass tubes. All blood samples were immediately placed on ice and transferred to the laboratory where they were centrifuged for 10 minutes at 3000 rpm. Plasma was removed and duplicate samples frozen at –80°C until assay. Both parent drugs and their metabolites tested were stable under these conditions for at least 9 months.

Drugs and their metabolites were extracted from plasma simultaneously along with an internal standard (proguanil). Plasma samples (1mL) in acid-cleaned glass centrifuge tubes were spiked with 100 ng/mL of proguanil (as an internal standard). Thereafter, 100 μL of 1M NaOH was added followed by vigorous vortexing. Extraction agent (methylene chloride: isopropanol; 45:1) was added (5mL) and vortexed for 30 seconds. Tubes were then centrifuged for 10 minutes at 3000 rpm. A 4 mL aliquot of the organic phase was removed into a clean glass tube and the solvent removed under vacuum in a centrifugal vacuum concentrator. Samples were reconstituted in 250 μL

of 32% acetonitrile/0.03M heptane sulphonic acid and injected onto the HPLC. Recovery was greater than 95% for both drugs and metabolites.

2.10.3 Assay methods

Assays were performed by trained Laboratory staff of the Department of Clinical Pharmacology, University of Cape Town.

CPG and CCG were assayed according to a modified method of Petersen *et al* (1991). DDS and MADDS were assayed according to a modified method of Jones and Overnell (1979) [22,27]. All drugs and their metabolites were assayed by high performance liquid chromatography (HPLC) after extraction from plasma. Each assay was validated prior to commencement of the study.

All drugs were separated on a Prodigy 5 μm ODS 2 HPLC column (4.6 x 15 mm, Phenomonex)

CPG

CPG was eluted isocratically with a mobile phase of 31% acetonitrile, 0.1% trifluoroacetic acid. Detection was at 250 nm and 60 μL of sample was injected onto the column. CPG eluted at 7.45 minutes and proguanil at 3.38 minutes. A representative chromatogram is given in **(Fig 1)** A standard curve was set up in the range 10-500 ng/mL and the lower limit of quantification was 5 ng/mL. The coefficient of variation was 8.2%.

CCG

CCG was eluted with a mobile phase of 32% acetonitrile, 0.03M heptane sulphonic acid, pH 2.0. Detection was at 250 nm and 100 μL of sample was injected onto the

column. CCG eluted at 6.01 minutes. A representative chromatogram is given in **(Fig 2)** A standard curve was set up in the range 5-100 ng/mL and the lower limit of quantification was 2 ng/mL. The coefficient of variation was 10.2%.

DDS/MADDS

DDS was eluted isocratically with a mobile phase of 25% acetonitrile, 0.01% trifluoroacetic acid. Detection was at 280 nm and 25 µL of sample was injected onto the column. DDS eluted at 3.18 minutes and MADDS at 4.04 minutes. A representative chromatogram is given in **(Fig 3)** A standard curve was set up in the range 0.05-5 µg/mL and the lower limit of quantification was 0.02 µg/mL. The coefficient of variation was 7.6%.

2.10.4 Pharmacokinetic analysis

Plasma concentrations of CPG, CCG, DDS and MADDS were summarised at each sample point by dose of CPG (Phase 1 to 4)

Individual subject profiles of plasma concentrations of CPG, CCG, DDS and MADDS were determined for each dose of CPG (Phase 1 to 4) and a mean profile of plasma CPG, CCG, DDS and MADDS versus time was produced for each dose of CPG (Phase 1 to 4). **(SEE SECTION 6)**

The following pharmacokinetic parameters were derived for CPG, DDS and MADDS for each dose of CPG (Phase 1 to 4) on day 0.

AUC_{0-t}	Area under the concentration-time curve over the sampling period
C_{max}	Peak serum concentration from the measured data
t_{max}	Time to peak serum concentration from the measured data

k Elimination rate constant

Area under the curve was calculated using the linear trapezoidal rule.

Only three sampling points were utilised on days 1 and 2, making estimation of parameters requiring use of the elimination phase data points very unreliable. Only pharmacokinetic parameters obtainable from the measured data have been presented for days 1 and 2: AUC_{0-t}, C_{max} and t_{max}.

CCG levels appeared to be in the absorption phase only over the sampling period on all days. Only pharmacokinetic parameters obtainable from the measured data have been presented for days 0, 1 and 2: AUC_{0-t}, C_{max} and t_{max}.

Each of the pharmacokinetic parameters for CPG, CCG, DDS and MADDS were summarised. The median was used in place of the mean and standard deviation for t_{max}.

A graph of AUC_{0-t} for CCG for each subject versus dose of CPG was produced.

2.11 Statistical Evaluation

Statistical analysis was performed using Sigma Plot ® Scientific Graphing Software Version 2.01 Copyright (1986-1994), and Graph Pad Prism ® Version 2.01 Copyright (1994,1995,1996), Graph Pad Software Incorporated. Statistical summary followed the intention-to-treat principle.

2.11.1 Populations/Data Sets to be Evaluated

The intention-to-treat (ITT) population for the study was all subjects entered into the study, who received at least one dose of CPG and one dose of DDS.

Safety Population

The population for the assessment of safety was all subjects who received at least one dose of CPG and one dose of DDS.

2.11.2 Safety Evaluations

Adverse Experiences

Non-serious adverse events were coded to the preferred term and body system organ class using the WHO Adverse Reaction Terminology Dictionary.

3 Study Population

3.1 Subject Disposition

3.1.1 Number and Distribution of Subjects

Twelve male subjects were recruited into the study and all completed.

3.1.2 Number of Subjects Present at Each Visit

All 12 subjects participated in each of the four dosing Phases.

3.1.3 Withdrawal Reasons

All 12 subjects completed the study, participating in each rising-dose Phase.

3.2 Protocol Violations

All 12 subjects took at least one dose of CPG and at least one dose of dapsone and are considered evaluable for safety. Since all 12 subjects took all doses in each of the four Phases, the safety population for each Phase is based on 12 subjects.

One subject reported an abnormality in the medical history. This subject reported hayfever "sometimes in the summer" for the 'other' category.

None of the deviations were considered to be major violations.

3.3 Demographic and Baseline Characteristics

3.3.1 Demographic Characteristics

The mean age was 25.8 years (SD: 4.2), with the youngest subject being 19 years and the oldest being 35 years.

3.3.2 Baseline Characteristics

Physical examination

All 12 subjects recorded normal physical examination for each of the body systems: cardiovascular, respiratory, central nervous system, skin, gastrointestinal, musculoskeletal, ENT, eyes and other.

ECG at screening

Mean ventricular rate was 65.1 bpm (SD: 14.6), mean PR interval was 153.3 ms (SD: 21.6) and mean QRS interval was 98.3 ms (SD: 6.7). Both QT and corrected QT (QTc) were recorded. Mean QT was 402.0 ms (SD: 40.9) and mean QTc was 407.6 ms (SD: 13.9).

3.4 Medical History

All 12 subjects recorded normal medical history and concomitant conditions for the following body systems: cardiovascular, respiratory, central nervous system, skin, gastrointestinal, musculoskeletal, ENT and eyes. One subject reported an 'other' abnormality, which was hayfever sometimes in the summer.

3.5 Prior and Concomitant Medications

There were no prior or concomitant medications.

4 Efficacy Results

There were no efficacy assessments.

5 Safety Results

5.1 Extent of Exposure

All 12 subjects received 3 oral doses of CPG within each Phase and 3 oral doses of DDS, on days 0, 1 and 2.

5.2 Adverse Experiences

5.2.1 Concomitant Diseases or Drug Experiences

The number and types of adverse experiences are summarised below in Table 3

Table 3 Number (%) of subjects with most commonly occurring adverse experiences

Adverse experience	CPG Dose (mg/kg)			
	1.2 (N=12)	2.0 (N=12)	2.8 (N=12)	3.6 (N=12)
Headache	0	0	0	1 (8.3)
Anorexia	0	7 (58.3)	0	1 (8.3)
Abdominal Pain	0	0	0	1 (8.3)
Diarrhoea	0	0	2 (16.7)	1 (8.3)
Nausea	1 (8.3)	5 (41.7)	11 (91.7)	12 (100)
Stomatitis, Ulcerative	3 (25.0)	1 (8.3)	0	0
Tooth Disorder	0	0	0	1 (8.3)
Vomiting	0	2 (16.7)	7 (58.3)	11 (91.7)
Fever	0	0	1 (8.3)	0
Malaise	0	0	0	1 (8.3)

There were four subjects (33%) who experienced one or more events during Phase 1 (1.2 mg/kg), nine subjects (75%) during Phase 2 (2.0 mg/kg), 11 subjects (92%) during Phase 3 (2.8 mg/kg) and 12 subjects (100%) during Phase 4 (3.6 mg/kg).

Table 3 shows that there was an increase in adverse events in the study population as the dose of CPG was raised per phase. However, only nausea and vomiting achieved

statistical significant increases in occurrence ($p = < 0.001$, $r_s = 0.818$, Spearman Rank Correlation Coefficient), across all four phases.

5.2.2 Adverse Experiences Related to Study Medication

Only one event recorded during the study was considered unlikely to be related to the study drug. This was fever experienced by one subject on day 1 of Phase 3 (2.8 mg/kg). The relationship to study medication for four events was unknown: a subject Phase 4 malaise, anorexia, nausea and tooth disorder.

There were four subjects (33%) who experienced one or more related events during Phase 1 (1.2 mg/kg), nine subjects (75%) during Phase 2 (2.0 mg/kg), 11 subjects (92%) during Phase 3 (2.8 mg/kg) and 12 subjects (100%) during Phase 4 (3.6 mg/kg).

5.2.3 Adverse Experiences by Severity

The severity for four events was unknown. Details are given in Table 4 below

Table 4 Number (%) of severe adverse experiences (safety population)

Adverse Experience	CPG Dose (mg/kg)			
	1.2 (N=12)	2.0 (N=12)	2.8 (N=12)	3.6 (N=12)
Anorexia	0	0	0	1 (8.3)
Stomatitis, Ulcerative	1 (8.3)	0	0	0
Tooth Disorder	0	0	0	1 (8.3)
Malaise	0	0	0	1 (8.3)

5.3 Serious Adverse Experiences

There were no serious adverse experiences in this study.

5.4 Withdrawals Due to Adverse Experiences

There were no withdrawals due to adverse experiences.

5.5 Electrocardiographic Data (Annexure 1)

For ventricular rate, the change from day 0 to day 1 showed a mean increase for all four Phases: 1.0 bpm, 1.5 bpm, 0.8 bpm and 0.6 bpm for the 1.2 mg/kg, 2.0 mg/kg, 2.8 mg/kg and 3.6 mg/kg doses respectively. The change from day 0 to day 2 showed slightly higher mean increases for all four Phases: 1.3 bpm, 1.9 bpm, 5.7 bpm and 8.3 bpm for the 1.2 mg/kg, 2.0 mg/kg, 2.8 mg/kg and 3.6 mg/kg doses respectively. All

subjects showed an increase in the 3.6 mg/kg group: minimum increase was 3 bpm and the maximum increase was 16 bpm.

For PR interval, the change from day 0 to day 1 showed a mean decrease for the 1.2 mg/kg and 2.8 mg/kg groups: -5.7 ms and –7.3 ms respectively and a mean increase for the 2.0 mg/kg and 3.6 mg/kg groups: 3.0 ms and 1.4 ms respectively. The change from day 0 to day 2 showed a mean decrease for the 1.2 mg/kg, 2.0 mg/kg and 2.8 mg/kg groups: -7.7 ms, -1.7 ms and –1.7 ms respectively and a mean increase for the 3.6 mg/kg group: 1.3 ms.

For QRS duration, the change from day 0 to day 1 showed a mean decrease for all four Phases: -1.7 ms, -1.7 ms, -1.7 ms and –4.0 ms respectively. The change from day 0 to day 2 showed a mean decrease for all four Phases: -2.3 ms, -3.7 ms, -2.0 ms and –4.0 ms respectively.

For QT, the change from day 0 to day 1 showed a mean decrease for the 1.2 and 2.0 mg/kg groups (-10.7 ms and –7.0 ms respectively) and a mean increase for the 2.8 mg/kg and 3.6 mg/kg groups (1.0 ms and 3.2 ms respectively). The change from day 0 to day 2 showed a mean decrease for the 1.2, 2.8 and 3.6 mg/kg groups (-13.3 ms, -14.0 ms and –13.0 ms respectively) and a mean increase for the 2.0 mg/kg group (5.7 ms).

For QTc, the change from day 0 to day 1 showed a mean decrease for the 1.2 and 2.8 mg/kg groups (-5.8 ms and –0.3 ms respectively) and a mean increase for the 2.0 mg/kg and 3.6 mg/kg groups (0.3 ms and 1.2 ms respectively). The change from day 0 to day 2 showed a mean decrease for the 1.2 mg/kg group (-6.2 ms) and a mean increase for the 2.0, 2.8 and 3.6 mg/kg groups (12.2 ms, 2.2 ms and 7.8 ms respectively).

5.6 Vital Signs (Annexure 2)

Weight was only recorded on day 0 of each Phase and so only absolute values are summarised. The mean weight was very similar in each of the four Phases: 76.17 kg, 76.33 kg, 75.63 kg and 76.42 kg for the 1.2 mg/kg, 2.0 mg/kg, 2.8mg/kg and 3.6 mg/kg doses respectively.

For systolic blood pressure, the change from day 0 to days 1, 2 and 3 showed a mean increase on all days for the 1.2 mg/kg and 3.6 mg/kg doses and a mean decrease was observed on all days in the 2.0 mg/kg and 2.8 mg/kg groups. The smallest mean increase was observed for the 3.6-mg/kg group on day 1 (1.2 mmHg) and the largest mean increase was observed for the 1.2 mg/kg group on day 2 (5.4 mmHg). Individual subject increases did not exceed 20 mmHg. The smallest mean decrease was observed for the 2.0-mg/kg group on day 2 (-0.4 mmHg) and the largest mean decrease was observed for the 2.0 mg/kg group on day 1 (-3.1 mmHg). Individual subject decreases did not exceed 26 mmHg.

For diastolic blood pressure, the change from day 0 to days 1, 2 and 3 showed a mean decrease for all dose groups on all days, except for the 3.6 mg/kg group on day 2 when a mean increase of 2.0 mmHg was observed. The smallest mean decrease was observed for the 3.6-mg/kg group on day 3 (-0.1 mmHg) and the largest mean decrease was observed for the 2.8 mg/kg group on day 3 (-5.3 mmHg). Individual subject increases did not exceed 20 mmHg and individual subject decreases did not exceed 24 mmHg.

For heart rate, the change from day 0 to days 1, 2 and 3 showed a mean decrease on all days for the 1.2 mg/kg and 2.0 mg/kg doses and a mean increase was observed on all days in the 2.8 mg/kg group. The smallest mean decrease was observed for the 1.2 mg/kg group on day 1 (-2.8 bpm) and the largest mean decrease was observed for the 2.0 mg/kg group on day 2 (-4.9 bpm). The mean increases observed for the 2.8 mg/kg group ranged from 3.5 bpm to 5.1 bpm. The 3.6 mg/kg group did not show a consistent pattern, with no change on day 1, a mean decrease of –1.2 bpm on day 2 and no results on day 3.

For temperature, the change from day 0 to days 1, 2 and 3 showed a mean increase on all days for the 1.2 mg/kg and 3.6 mg/kg doses and a mean decrease was observed on all

days in the 2.8 mg/kg group. The smallest mean increase was observed on day 2 for the 1.2 mg/kg and 3.6 mg/kg groups (0.18 °C) and the largest mean increase was observed for the 3.6 mg/kg group on day 3 (0.65 °C). Individual subject increases did not exceed 1.3 °C. The mean decreases observed for the 2.8 mg/kg group ranged from –0.08 °C to –0.32 °C. Individual subject decreases for all groups did not exceed -1.4 °C.

5.7 Medical History and Concomitant Conditions During Each Phase

In the 1.2 mg/kg group, abnormal concomitant conditions were noted for the 'other' category on day 1 (2 subjects, 17%), day 2 (3 subjects, 25%) and day 3 (1 subject, 8%). No other abnormalities were noted.

In the 2.0 mg/kg group, abnormal concomitant conditions was noted for the 'gastrointestinal' category on day 2 (3 subjects, 25%) and day 3 (7 subjects, 58%) and the 'other' category on day 1 (1 subject, 8%). No other abnormalities were noted.

In the 2.8 mg/kg group, abnormal concomitant conditions were noted for the 'gastrointestinal' category on day 1 (1 subject, 8%), day 2 (2 subjects, 17%) and day 3 (3 subjects, 25%). No other abnormalities were noted.

In the 3.6 mg/kg group, abnormal concomitant conditions were noted for the 'gastrointestinal' category on day 1 (8 subjects, 67%), day 2 (10 subjects, 83%) and day 3 (10 subjects, 83%). No other abnormalities were noted.

5.8 Physical Examination During Each Phase

In the 1.2 mg/kg group, abnormal physical examination was noted for the 'other' category on day 1 (2 subjects, 17%), day 2 (3 subjects, 25%) and day 3 (1 subject, 8%). No other abnormalities were noted.

In the 2.0 mg/kg group, abnormal physical examination was noted for the 'other' category on day 1 (1 subject, 8%) and day 2 (1 subject, 8%). No other abnormalities were noted.

In the 2.8 and 3.6 mg/kg groups, no abnormal physical examinations were noted for any category on any day.

5.9 Laboratory Tests

There were dose-related increases in albumin, bilirubin (total) and bilirubin (unconjugated) and dose -related decreases in haematocrit and haptoglobin. None of

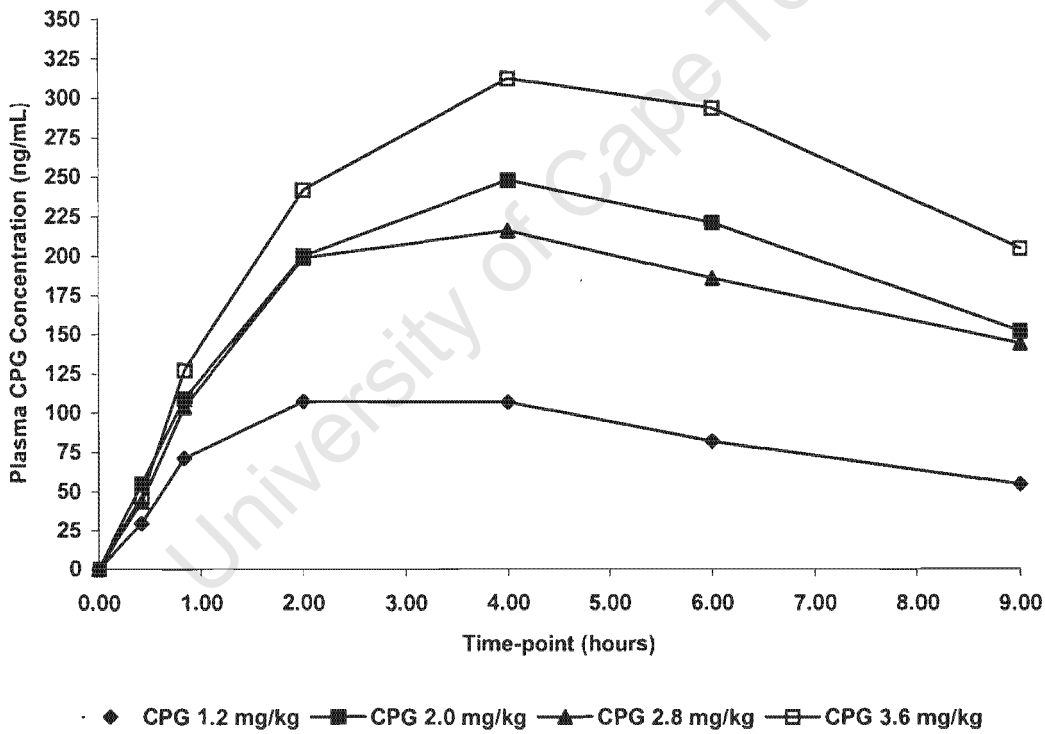
the changes within any individual subject were considered to be clinically significant by the Investigator. No remarkable changes were seen in any of the other laboratory parameters assessed. The haematological changes noted above were considered to be due to methaemoglobin formation associated with dapsone treatment.

6 Pharmacokinetic Evaluation

6.1 Chlorproguanil

Mean subject profiles are presented in Figure 6.1 below.

Figure 6.1 Mean 9-hour Plasma Profiles for CPG at Each Study Dose (Day 0)



The plasma concentration of CPG was zero for the pre-dose sample on day 0 of each phase. The plasma concentration of CPG was above zero for all subjects for the pre-dose sample on day 1 for the 2.8 mg/kg and 3.6 mg/kg groups. Two subjects in the 1.2 mg/kg and 2.0 mg/kg had zero concentration for the pre-dose sample on day 1. On day 2 the plasma concentration was above zero for all subjects pre-dose in all dose groups. The trough levels were above zero in all dose groups on days 3 and 4, but had returned to zero for some subjects in the 1.2 mg/kg and 2.0 mg/kg groups on day 5. Trough levels were above zero for all subjects on day 5 in the 2.8 mg/kg and 3.6 mg/kg groups.

The mean AUC_{0-t} did not increase linearly with dose of CPG on day 0 of each Phase: 710.39 ng.h/ml (± 216.32), 1692.24 ng.h/ml (± 368.63), 1520.77 ng.h/ml (± 295.68) and 2154.89 ng.h/ml (± 421.78) for the 1.2, 2.0, 2.8 and 3.6 mg/kg groups respectively.

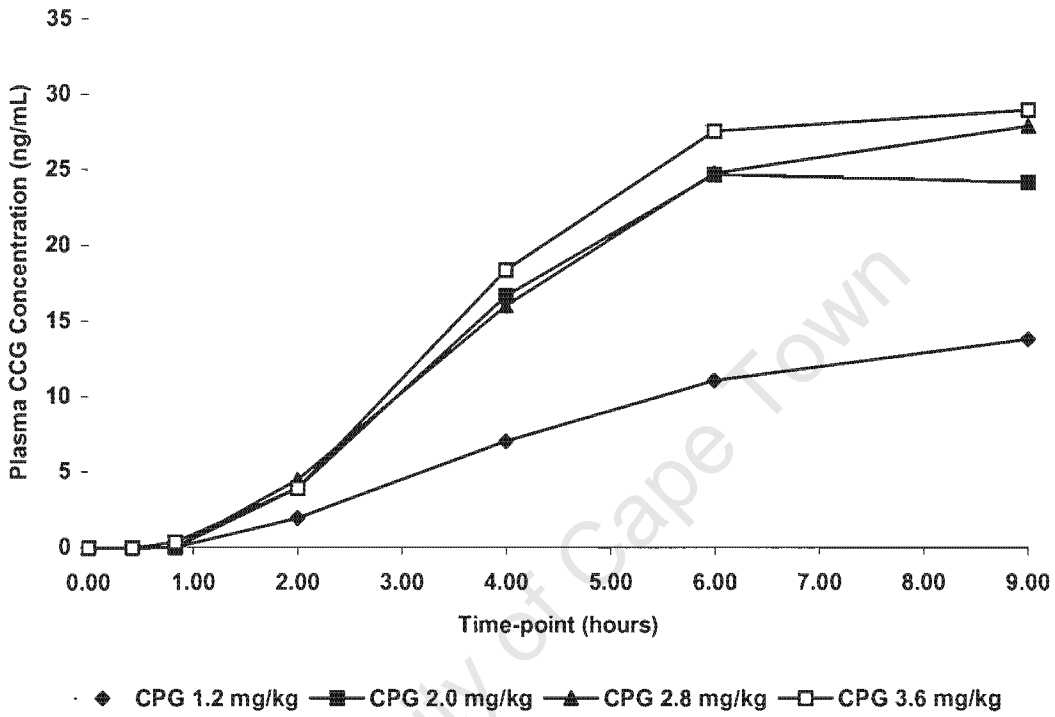
Within each phase the mean AUC_{0-t} increased slightly from day 0 to day 2 due to small residual drug from the previous days dose.

None of the subjects vomited on any day of Phase 1 (1.2 mg/kg), or on day 0 of subsequent Phases. Only one subject vomited on day 1 of Phase 2, none on day 1 of Phase 3 and 5 on day 1 of Phase 4. Two subjects vomited on day 2 of Phase 2, 7 on day 2 of Phase 3 and 11 on day 2 of Phase 4. With the exception of Phase 2 day 2, the AUC_{0-t} for CPG observed for the subjects who vomited does appear on average to be lower than observed for the subjects who did not vomit.

6.2 Chlorcycloguanil

Mean subject profiles are presented in Figure 6.2 below.

Figure 6.2 Mean 9-hour Plasma Profiles for CCG at Each Study Dose (Day 0)



The plasma concentration of CCG was zero for both the pre-dose and 25 minutes post-dose samples on day 0 of each Phase. The plasma concentration of CCG was above zero for all subjects for the pre-dose sample on day 1 for the 1.2 mg/kg, 2.8 mg/kg and 3.6 mg/kg groups. Three subjects in the 2.0-mg/kg group had zero concentration for the pre-dose sample on day 1. On day 2 the plasma concentration was above zero for all subjects pre-dose in all dose groups. The trough levels were above zero for all subjects in all dose groups on days 3, 4 and 5.

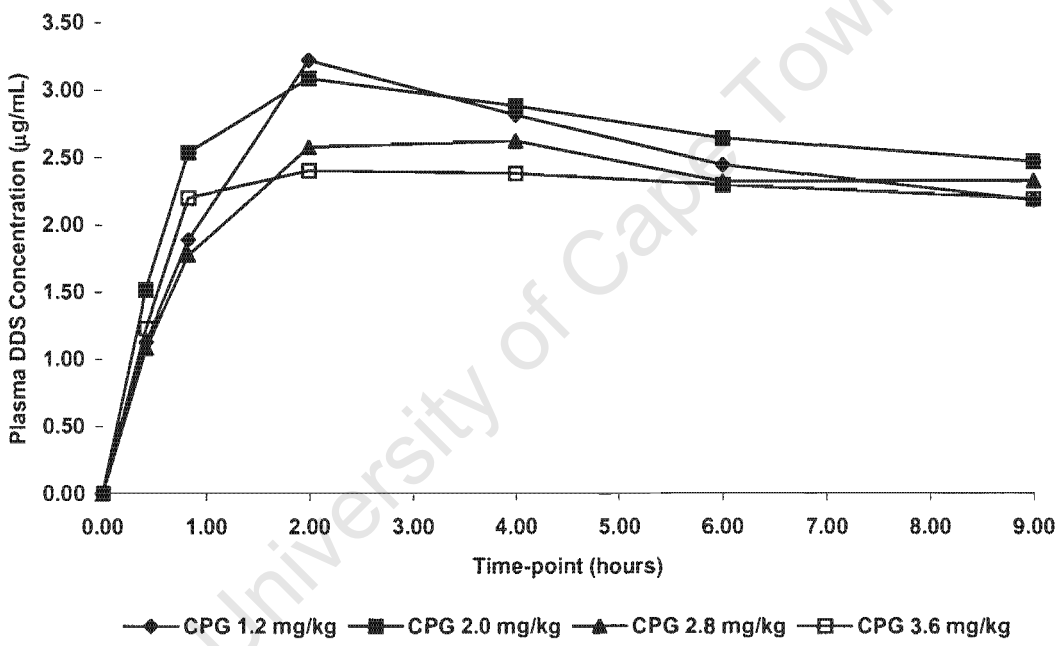
The mean AUC_{0-t} did not increase linearly with dose of CPG on day 0 of each Phase: 76.24 ng.h/ml (± 41.54), 137.50 ng.h/ml (± 46.63), 142.46 ng.h/ml (± 55.16) and 154.82 ng.h/ml (± 55.44) for the 1.2, 2.0, 2.8 and 3.6 mg/kg groups respectively.

Although the mean AUC_{0-t} increased with increasing dose of CPG, the largest increase was observed between the 1.2 mg/kg and 2.0 mg/kg doses. Only very slight average increases were observed for subsequent doses. The plasma levels of CCG may not have reached the true peak (Cmax) during the observed sampling period for many subjects as t_{max} was observed to be 9 hours (the last sample point) for many of the subjects.

6.3 Dapsone

Mean subject profiles are presented in Figure 6.3.

Figure 6.3 Mean 9-hour Plasma Profiles for DDS at Each Study Dose (Day 0)



The plasma concentration of DDS was zero for the pre-dose sample on day 0 of each Phase. The plasma concentration of DDS was above zero for all subjects for the pre-dose sample on day 1 for all groups: 1.2 mg/kg, 2.0 mg/kg, 2.8 mg/kg and 3.6 mg/kg. On day 2 the plasma concentration was above zero for all subjects pre-dose in all dose groups. The trough levels were above zero for all subjects in all dose groups on days 3, 4 and 5.

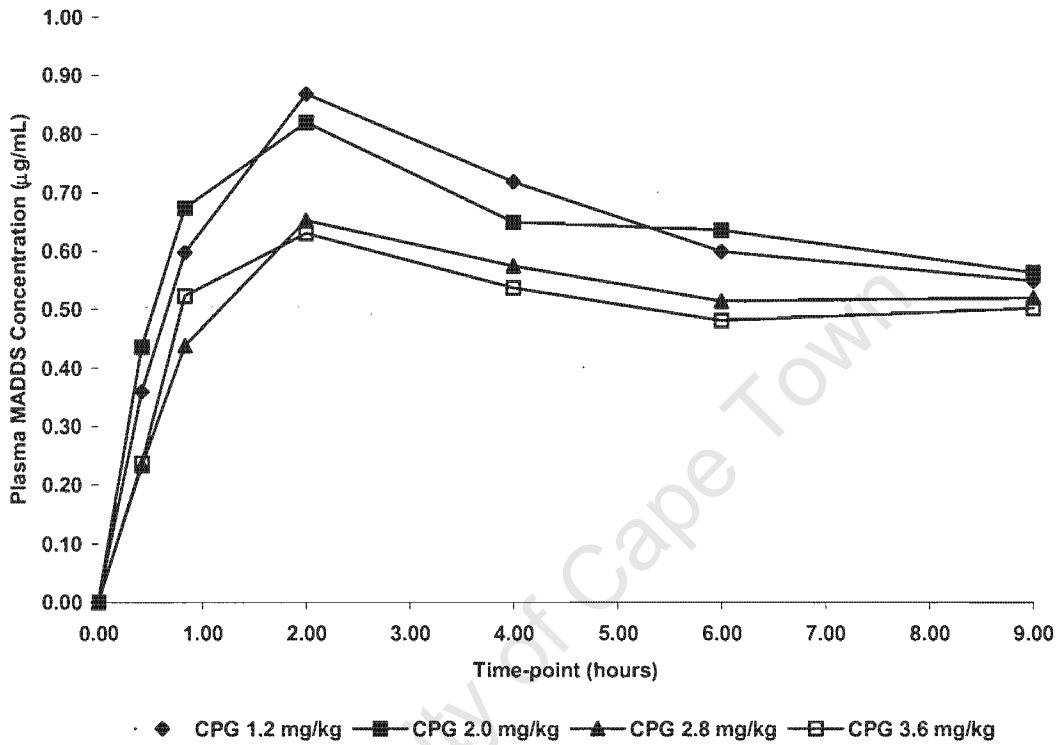
The mean AUC_{0-t} on day 0 of each Phase was 21.96 μg.h/ml (± 6.94), 23.56 μg.h/ml (± 3.42), 20.42 μg.h/ml (± 4.21) and 19.72 μg.h/ml (± 5.17) for the 1.2, 2.0, 2.8 and 3.6 mg/kg CPG groups respectively.

None of the subjects vomited on any day of Phase 1 (1.2 mg/kg), or on day 0 of subsequent Phases. Only one subject vomited on day 1 of Phase 2, none on day 1 of Phase 3 and 5 on day 1 of Phase 4. Two subjects vomited on day 2 of Phase 2, 7 on day 2 of Phase 3 and 11 on day 2 of Phase 4. With the exception of Phase 2 day 2, the AUC_{0-t} for dapsone observed for the subjects who vomited does appear on average to be lower than observed for the subjects who did not vomit.

6.4 Mono acetyl Dapsone

Mean subject profiles are presented in Figure 6.4.

Figure 6.4 Mean 9-hour Plasma Profiles for MADDS at Each Study Dose (Day 0)



The plasma concentration of MADDS was zero for the pre-dose sample on day 0 of each Phase. The plasma concentration of MADDS was above zero for all subjects for the pre-dose sample on day 1 for all dose groups: 1.2 mg/kg, 2.0 mg/kg, 2.8 mg/kg and 3.6 mg/kg. On day 2 the plasma concentration was above zero for all subjects pre-dose in all dose groups. The trough levels were above zero in all dose groups on days 3 and 4.

Trough levels were above zero for all subjects on day 5 in the 1.2 mg/kg, 2.8 mg/kg and 3.6 mg/kg groups.

The mean AUC_{0-t} on day 0 of each Phase was 5.68 μg.h/ml (± 4.85), 5.75 μg.h/ml (± 3.72), 4.70 μg.h/ml (± 3.29) and 4.51 μg.h/ml (± 3.15) for the 1.2, 2.0, 2.8 and 3.6 mg/kg groups respectively.

6.5 Summary of Pharmacokinetic Parameters

The pharmacokinetic parameters for CPG, CCG, DDS and MADDS are summarised in Table 5 below.

As expected CPG showed dose-related increases in the pharmacokinetic parameters AUC and Cmax. CCG also showed dose-related increases but the increase was not linear with respect to the dose of parent compound administered. C_{max} and t_{max} values for CCG are approximate as conversion of CPG to CCG was incomplete at 9 hours in a number of subjects. Detectable and increasing levels of CPG and CCG were present pre-dose on Days 1, 2 respectively and also on Day 3. Levels started to decrease but were still detectable on Days 4 and 5 at all dose levels of CPG.

For DDS and MADDS, C_{max} values tended to decrease with increasing dose of CPG. Elimination was slow in both and incomplete by the end of the 9-hour sampling period. As with CPG and CCG there were detectable and increasing levels of drug and metabolite present pre-dose on Days 1 and 2, and on Day 3. These levels were decreasing by Days 4 and 5.

Table 5 Summary of Pharmacokinetic Parameters

Analyte	CPG dose mg/kg	Pharmacokinetic Parameter (mean/median at Day 0) AUC_{0-t} ng.h/mL(±SD)		C_{max} ng/mL(±SD)		t_{max} (hours)	k
CPG							
	1.2	710.4	(216.3)	107.5	(33.1)	2.0	0.122
	2.0	1692.2	(368.6)	258.9	(44.9)	4.0	0.111
	2.8	1520.8	(295.7)	231.4	(45.3)	4.0	0.094
	3.6	2154.	(421.8)	330.2	(51.5)	4.0	0.112
CCG							
	1.2	76.3	(41.5)	16.5	(7.2)	9.0	NR
	2.0	137.5	(46.6)	25.7	(8.3)	6.0	NR
	2.8	142.4	(55.1)	28.6	(9.7)	9.0	NR
	3.6	154.8	(55.4)	30.7	(9.8)	9.0	NR
DDS							
	1.2	21962	(6935)	3268	(981)	2.0	0.062
	2.0	23555	(3422)	3313	(575)	2.0	0.051
	2.8	20423	(4214)	3047	(575)	2.0	0.042
	3.6	19724	(5173)	2946	(605)	2.0	0.045
MADDS							
	1.2	5682	(4851)	902	(646)	2.0	0.076
	2.0	5745	(3722)	875	(593)	2.0	0.052
	2.8	4696	(3290)	727	(528)	2.0	0.043
	3.6	4511	(3150)	727	(436)	2.0	0.047

7. Discussion

In sub-Saharan Africa the burden of disease due to malaria each year is enormous, with an estimated 1 million deaths annually, of whom 700 000 being children aged 0-4 years.

Thirty three million Disability Adjusted Life Years (DALYs) were lost in sub-Saharan Africa in 1999 due to malaria, which is over 11% of total DALYs lost.[9]

Resistance to antimalarial drugs affects the costs borne by households and the health delivery system. Resistance to chloroquine, once a safe, cheap and effective antimalarial drug is now common throughout malaria endemic areas of Africa. Pyrimethamine resistance has occurred over many years, probably due to mass drug use and chemoprophylaxis. Resistance to the combination of sulfadoxine and pyrimethamine (SP) was recorded in the 1970s. The long half-life of SP allowed for a single dose, with the advantage of increased compliance. However, it has been suggested that the long half-life of SP has contributed to selection pressure and drug resistance. An appropriate antimalarial agent will minimise the increasing cost of treatment to the health delivery system. The shorter half-life combination agents, chlorproguanil/dapsone, may exert less selection pressure and thus reduce the development of resistance, compared to SP.

The use of less effective agents will result in more treatment failures due to poor drug efficacy or compliance. Consequently treatment costs will rise because of an increase in consultations, the treatment of complications and the overall effects of a greater malaria morbidity, mortality and debility.

Time lost due to an uncomplicated malaria illness in a semi-immune person will be on average 3.5 days. Time lost in caring for a child with uncomplicated malaria will be

on average 5 days.[10] The indirect effects on the child due to absence from school may be significant. The financial impact of the time lost from work will be linked to the seasonality of malaria and the morbidity producing a decrease in potential productivity of the individual. If, as a result of SP resistance, the number of treatment failures increases, then the duration of time lost from work will increase. Lack of clinical remission or lack of clinical cure will require time to seek further therapy and possible referral to a more specialised unit. This will take longer if a patient requires hospitalisation for severe disease.

The aim of the study was to define the dose-limiting toxicity of CPG, given in combination with a fixed dose of DDS, in healthy volunteers. All the study subjects were extensive metabolisers of CPG. A secondary aim was to determine the relationship between the dose of CPG and the area under the curve of its active metabolite, CCG.

A total of 12 healthy male volunteers were entered into the study and each subject completed all four Phases. During each succeeding Phase a sequentially higher dose of CPG was administered for the first three days, according to the following dosage schedule: 1.2mg/kg, 2.0mg/kg, 2.8mg/kg and 3.6mg/kg.

AUC for the metabolite, CCG was reported as AUC_{0-t} because the majority of subjects had not reached t_{max} values at 9 hours, which was the last sample point for the study day, indicating that the sampling was covering only the metabolite formation phase and gave no information regarding elimination. The mean AUC_{0-t} values on day 0 of each Phase increased in general, but not in a linear manner with increase in dose of CPG. The largest increase was observed between the 1.2 mg/kg and 2.0 mg/kg dose.

At higher CPG doses the increases in CCG levels were disproportionately small and clearly non-linear. The variability observed between AUC_{0-t} values on each study day within a Phase was quite large, particularly on day 2 of Phase 2.

A plateau of CPG and, more notably, of CCG levels occurred above 2mg/kg. While it would appear that saturation of either absorption of CPG or metabolism to CCG had occurred, this explanation seems implausible given the large capacity of the enzyme systems involved[1]. However, it is more likely that this observation arises from the high variability of the data due to a reduced sample size.

For DDS and MADDS, C_{max} values tended to decrease with increasing dose of CPG. The reduction in DDS and MADDS peak levels was small when the CPG dose was increased to 2.0 mg/kg, but was more marked as the dose of CPG was increased. Elimination was slow for both DDS and MADDS and the estimates of AUC were not reliable beyond nine hours.

The incidence of drug related adverse experiences increased with the increase in CPG dose, particularly the incidence of nausea and vomiting. For the 3.6 mg/kg group there were 42 reports of nausea and every subject experienced nausea on one or more of the study days in Phase 4. Some subjects experienced more than one episode per day. There were some dose-related changes in laboratory parameters, although none of these was considered to be clinically significant within any individual subject. The changes are consistent with haemolysis and are known effects of dapsone.

Doses of CPG above 2.0 mg/kg do not appear to be associated with any additional benefits in terms of systemic exposure to the active metabolite CCG. Furthermore, increasing the CPG dose beyond this slightly lowers circulating DDS and MADDS levels. Coupled with the fact that doses above 2.0 mg/kg are associated with a higher

incidence of unwanted effects such as nausea and vomiting, a dose of CPG of 2.0 mg/kg is considered the optimal dose for the CPG/DDS combination.

There is an urgent need for effective drugs for the management of malaria that will be accessible and affordable to countries and individuals exposed to the problems of resistance.

The priority is to develop new agents cheaply, by establishing a reasonable cost of treatment and working towards achieving this target in the most economical way.

Malaria treatment in Africa is dependent on the rapid treatment of mild disease, usually with the cheap and effective drug chloroquine.[3,8,10,12] However, chloroquine resistance in the malarial parasite is high, while the long half-life alternative SP tends to encourage the development of resistance.[3,8,1012] Short half-life antifolates such as chlorproguanil/dapsone could overcome this problem. Studies in Africa have found this combination to be both safe and effective, especially in children.[3,8]

Our findings suggest a definite therapeutic advantage for the chlorproguanil/dapsone combination in the treatment of uncomplicated malaria. Its shorter half-life and acceptable tolerability suggest that it may be a better alternative to SP.

Future direction would include additional studies, examining the clinical and parasitological efficacy of chlorproguanil/dapsone in a suitably large patient population.

The recommended dose should be 2.0 mg/kg/day of chlorproguanil in combination with 2.4 mg/kg/day dapsone for three days.

Studies should include a double-blind comparison with SP as treatment for uncomplicated malaria in adults and children.

If these studies show acceptable clinical and parasitological efficacy as well as safety, a sponsor must be secured for future production and marketing of the agent after the necessary regulatory requirements have been satisfied.

8. Conclusions

- Doses of CPG above 2.0 mg/kg in combination with 2.4 mg/kg DDS are associated with a marked increase in the incidence of unwanted effects such as nausea and vomiting.

- Increasing CPG dose increases CCG levels, but the effect is not linear.

- The apparent plateau of CCG levels is achieved at a dose of CPG of 2mg/kg without significant additional plasma concentrations being achieved with higher CPG doses. Any marginal benefits are counterbalanced by side effects.

- 2.0mg/kg CPG daily for 3 days is the recommended as the dose of choice in combination with 2.4mg/kg DDS for future studies.

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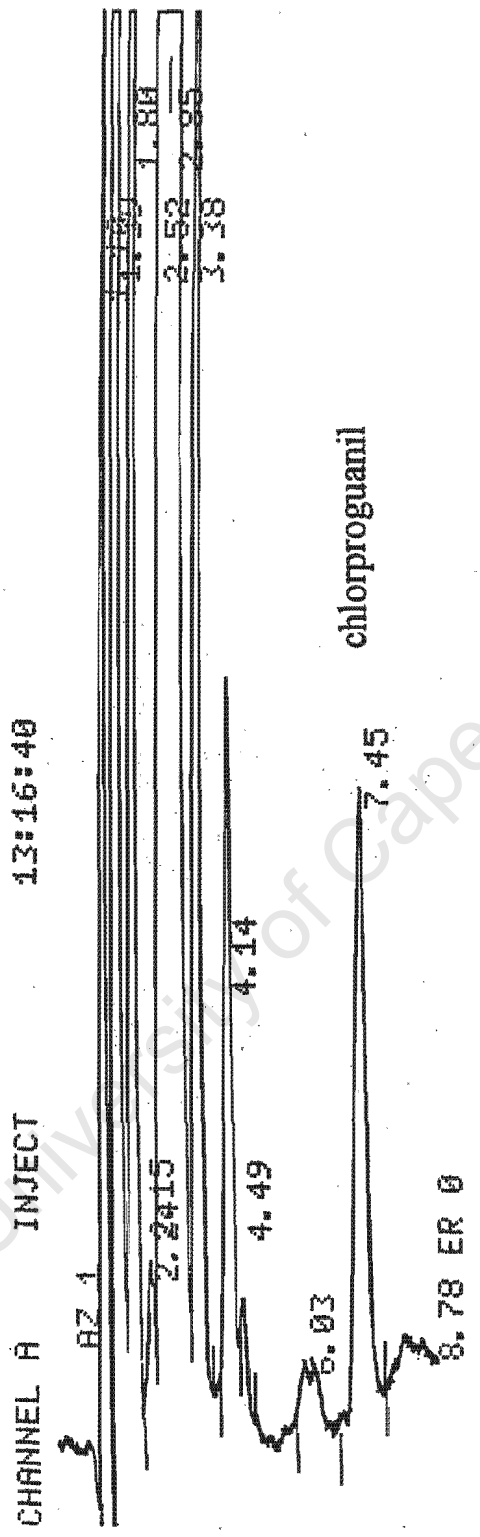

Figure 1 A representative chromatogram of chlorproguanil (100 ng/ml) extracted from plasma

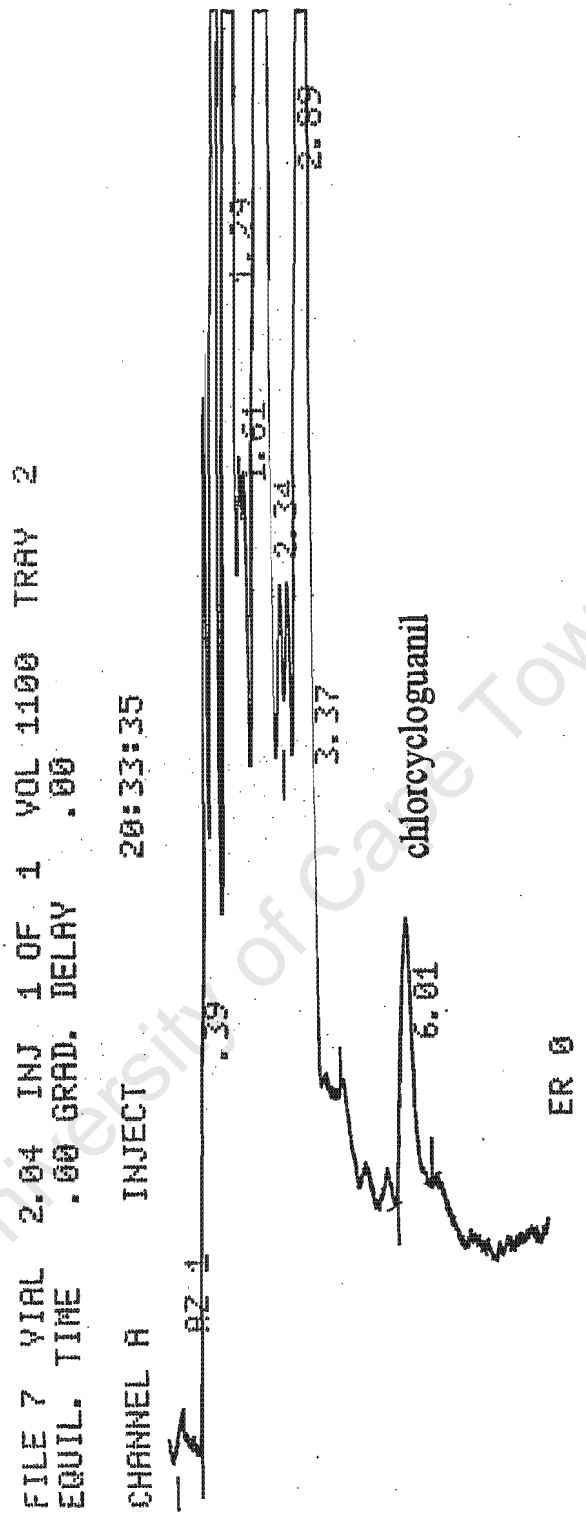

Figure 2 A representative chromatogram of chlorcycloguanil (20 ng/ml) extracted from plasma

Figure 3 A representative chromatogram of dapsone (3 ug/ml) extracted from plasma

ANNEXURE 1

Change From Baseline in ECG During the Study: Ventricular Rate (bpm)

Day		Dose of CPG 1.2 mg/kg	2.0 mg/kg	2.8 mg/kg	3.6 mg/kg
1	N	12	12	12	5
	Mean	1.0	1.5	0.8	0.6
	SD	9.2	6.5	10.1	11.8
	Median	3.5	1.5	1.0	1.0
	Minimum	-18	-12	-15	-13
	Maximum	14	10	26	17
2	N	12	12	12	4
	Mean	1.3	1.9	5.7	8.3
	SD	9.7	5.1	5.8	5.5
	Median	2.0	4.0	7.5	7.0
	Minimum	-18	-9	-4	3
	Maximum	14	8	18	16

ANNEXURE 1

Change From Baseline in ECG During the Study: PR interval (ms)

Day		Dose of CPG 1.2 mg/kg	2.0 mg/kg	2.8 mg/kg	3.6 mg/kg
1	N	12	12	12	5
	Mean	-5.7	3.0	-7.3	1.4
	SD	13.3	8.9	10.2	6.1
	Median	-4.0	2.0	-6.0	0.0
	Minimum	-28	-16	-24	-4
	Maximum	20	16	12	12
2	N	12	12	12	3
	Mean	-7.7	-1.7	-1.7	1.3
	SD	12.7	14.1	9.3	10.1
	Median	-8.0	-2.0	-4.0	0.0
	Minimum	-36	-32	-20	-8
	Maximum	12	28	16	12

ANNEXURE 1

Change From Baseline in ECG During the Study: QRS duration (ms)

Day		Dose of CPG 1.2 mg/kg	2.0 mg/kg	2.8 mg/kg	3.6 mg/kg
1	N	12	12	12	5
	Mean	-1.7	-1.7	-1.7	-4.0
	SD	6.0	6.3	3.2	2.8
	Median	0.0	0.0	0.0	-4.0
	Minimum	-12	-12	-8	-8
	Maximum	4	12	4	0
2	N	12	12	12	4
	Mean	-2.3	-3.7	-2.0	-4.0
	SD	5.0	7.1	3.2	3.3
	Median	0.0	-4.0	-2.0	-4.0
	Minimum	-12	-16	-8	-8
	Maximum	4	8	4	0

ANNEXURE 1

Change From Baseline in ECG During the Study: QT (ms)

Day		Dose of CPG 1.2 mg/kg	2.0 mg/kg	2.8 mg/kg	3.6 mg/kg
1	N	12	12	12	5
	Mean	-10.7	-7.0	1.0	3.2
	SD	21.9	28.1	28.0	28.6
	Median	-16.0	2.0	0.0	4.0
	Minimum	-40	-76	-60	-36
	Maximum	24	24	64	44
2	N	12	12	12	4
	Mean	-13.3	5.7	-14.0	-13.0
	SD	22.8	43.9	16.3	6.8
	Median	-12.0	-2.0	-10.0	-14.0
	Minimum	-48	-52	-40	-20
	Maximum	24	128	4	-4

ANNEXURE 1

Change From Baseline in ECG During the Study: QTc (ms)

Day		Dose of CPG 1.2 mg/kg	2.0 mg/kg	2.8 mg/kg	3.6 mg/kg
1	N	12	12	12	5
	Mean	-5.8	0.3	-0.3	1.2
	SD	15.5	15.0	11.3	12.7
	Median	-1.5	4.0	0.0	4.0
	Minimum	-40	-19	-25	-20
	Maximum	12	24	18	14
2	N	12	12	12	4
	Mean	-6.2	12.2	2.2	7.8
	SD	19.4	27.5	7.6	13.4
	Median	-4.0	7.0	0.5	11.0
	Minimum	-44	-22	-11	-10
	Maximum	20	88	16	19

ANNEXURE 2

Change From Day 0 in Vital Signs During the Study: Systolic Blood Pressure (mmHg)

Day		Dose of CPG 1.2 mg/kg	2.0 mg/kg	2.8 mg/kg	3.6 mg/kg
1	N	12	12	12	12
	Mean	3.6	-3.1	-2.3	1.2
	SD	10.9	9.2	9.9	12.9
	Median	2.0	-3.0	0.0	1.0
	Minimum	-14	-18	-20	-20
	Maximum	20	10	12	20
2	N	12	12	12	12
	Mean	5.4	-0.4	-2.0	2.6
	SD	11.1	10.3	10.8	7.1
	Median	5.0	3.0	-3.0	1.0
	Minimum	-14	-26	-20	-12
	Maximum	20	10	20	15
3	N	12	12	12	12
	Mean	4.3	-0.8	-1.9	5.1
	SD	8.6	8.6	8.7	11.8
	Median	4.5	0.0	-3.0	9.0
	Minimum	-12	-16	-14	-22
	Maximum	16	12	20	20

ANNEXURE 2

Change From Day 0 in Vital Signs During the Study: Diastolic Blood Pressure (mmHg)

Day		Dose of CPG 1.2 mg/kg	2.0 mg/kg	2.8 mg/kg	3.6 mg/kg
1	N	12	12	12	12
	Mean	-3.0	-2.4	-2.7	-0.4
	SD	7.4	6.0	9.7	14.6
	Median	-2.5	-2.0	-2.0	-3.0
	Minimum	-18	-18	-22	-24
	Maximum	7	8	10	20
2	N	12	12	12	12
	Mean	-0.6	-3.6	-3.2	2.0
	SD	8.0	5.1	7.8	8.3
	Median	-0.5	-3.0	-4.0	2.0
	Minimum	-13	-12	-12	-14
	Maximum	14	6	8	14
3	N	12	12	12	12
	Mean	-1.8	-3.8	-5.3	-0.1
	SD	7.0	4.3	6.1	10.5
	Median	-2.0	-3.0	-7.0	0.0
	Minimum	-12	-10	-14	-14
	Maximum	14	4	2	19

ANNEXURE 2

Change From Day 0 in Vital Signs During the Study: Heart Rate (bpm)

Day		Dose of CPG 1.2 mg/kg	2.0 mg/kg	2.8 mg/kg	3.6 mg/kg
1	N	12	12	11	12
	Mean	-2.8	-4.6	3.5	0.0
	SD	9.0	4.9	6.5	7.9
	Median	-3.0	-4.0	6.0	-2.0
	Minimum	-16	-12	-14	-12
	Maximum	12	2	8	12
2	N	12	12	11	12
	Mean	-4.0	-4.9	5.1	-1.2
	SD	9.3	4.1	7.6	6.2
	Median	-2.0	-5.0	8.0	0.0
	Minimum	-20	-12	-6	-10
	Maximum	6	0	14	10
3	N	12	12	11	0
	Mean	-3.3	-2.9	3.5	.
	SD	9.4	7.1	7.1	.
	Median	-0.5	-3.0	6.0	.
	Minimum	-21	-16	-6	.
	Maximum	7	10	12	.

ANNEXURE 2

Change From Day 0 in Vital Signs During the Study: Temperature (°C)

Day		Dose of CPG 1.2 mg/kg	2.0 mg/kg	2.8 mg/kg	3.6 mg/kg
1	N	12	12	12	12
	Mean	0.23	-0.05	-0.14	0.33
	SD	0.59	0.34	0.50	0.41
	Median	0.20	0.00	-0.15	0.45
	Minimum	-0.6	-0.8	-1.3	-0.2
	Maximum	0.9	0.4	0.8	1.0
2	N	12	12	12	12
	Mean	0.18	-0.02	-0.32	0.18
	SD	0.41	0.20	0.43	0.43
	Median	0.20	0.00	-0.25	0.20
	Minimum	-0.5	-0.4	-1.4	-0.4
	Maximum	0.8	0.3	0.2	1.0
3	N	12	12	12	11
	Mean	0.25	0.02	-0.08	0.65
	SD	0.43	0.22	0.39	0.46
	Median	0.35	0.00	0.00	0.70
	Minimum	-0.6	-0.4	-0.8	0.0
	Maximum	0.9	0.5	0.5	1.3