

Research Paper

EFFECT OF COMBINED QUININE AND CIPROFLOXACIN THERAPY IN EXPERIMENTAL MURINE PLASMODIASIS

ABSTRACT

The increasing spread of drug-resistant malaria parasite has necessitated the continuous search for even more effective malaria treatment including the combination of drugs known to have significant antimalarial potentials. Effort in this study was therefore, devoted to evaluating *in vivo*, antiplasmodial activity of combination of varying doses (4.3, 8.6, 12.9 mg/kg body weight) of quinine (a known antimalarial gradually losing relevance) with varying doses (5.0, 10.0, 15.0 mg/kg body weight) of ciprofloxacin (a fluoroquinolone commonly used to treat bacterial infections and has been shown to possess significant antimalarial activity both *in vitro* and *in vivo*) in *Plasmodium berghei* infected mice. Parasitological activity and survival of the animal were assessed over 21 days. Parasitemia in non-treated control mice peaked at 75% on day 9 and none survived by day 11. The lower dosages of quinine (4.3 and 8.6 mg/kg body weight) and ciprofloxacin (5.0 and 10.0 mg/kg body weight) were not efficient. However, the combination of 12.9 mg/kg body weight of quinine with 15 mg/kg body weight of ciprofloxacin achieved 87% reduction in parasitemia level and significantly reduced mortality in the infected animals compared with other treatment groups. The results from this study support the potential use of ciprofloxacin in combination with quinine for the treatment of resistant malaria.

Key words: Drug resistance, Combination, *Plasmodium berghei*, Quinine, Ciprofloxacin, Parasitaemia

INTRODUCTION

Malaria is a chronic parasitic disease of man transmitted exclusively through the bite of an infected *Anopheles* mosquito. It is known to be one of the oldest diseases of man especially in the tropics and is characterized by fever, anaemia and damage to some organs like liver and brain. Malaria currently, remains one of the major killer diseases of the world, especially in Sub-Saharan Africa (1).

The greatest problem the medical world has encountered with malaria is drug resistance and the situation seem not to be getting any better with reports from different parts of the world constantly pointing to a worsening situation. For example, the ever dependable quinine which has been used to treat malaria is now reported to be getting less effective over times in different parts of the world. Other drugs which are still effective like artemisin derivatives are too costly and cannot be used in some places like the developing countries on a mass scale (2). This is applicable even now.

Combination therapy with antimalarial drugs involves the simultaneous use of two or even more antimalarials which may have independent modes of action and therefore, different biochemical targets in the parasites. Currently, the guideline for the treatment of malaria includes among others, the use of one antimalarial plus antibiotics provided that there is adequate evidence of their efficacy and safety.(1b)

It is believed that besides the desired killing of the malarial parasites due to additive or synergistic action, these combinations also help in the reduction of the development of further resistance (4). Currently, a lot of effort is being made to find suitable combinations that can be used against these agents of destruction. For example, Atovaquone showed low activity in human malaria trials when used alone but when combined with malarone (Proguanil hydrochloride), it showed improved activity and the combination is therefore recommended for the treatment of uncomplicated malaria by WHO. The work of Andrade et al (2007) (10), also confirms the efficacy of the combination of ciprofloxacin with an antimalarial (Mefloquine) against *P. falciparum* compared to when each of the drugs were used alone.

Ciprofloxacin is a second generation, broad spectrum antibacterial agent. It belongs to the fluoroquinolone group of antibiotics and has been reported to be promising in the possibility of using it as an antimalarial but may have to be used at very high concentration to be able to achieve the required serum concentration for effectiveness (5). This and a few other reasons have discouraged the possible use of ciprofloxacin alone in the management of malaria. Instead, it is recommended for use in combination with some other antibacterials, especially those which are fast losing credibility as potent antimalarials e.g quinine. This will not only make it possible for a reasonable concentration of the antibacterial ciprofloxacin to be used but for quinine to continue to be relevant.

Quinine is a cinchona alkaloid known to belong to the aryl amino alcohol group of drugs (6). Always presented as a salt, quinine is known to have rapid schizonticidal action against intra erythrocytic malarial parasites. Quinine has been an antimalarial drug of choice over a long time even though, recently, it has been reported to lose its premium position to some other antimalarials. This may not be unconnected with the

challenge has been encountered using quinine, principal among which is poor tolerability and compliance with dosing regime.

This paper presents the report of the effect of combined quinine and ciprofloxacin therapy in plasmodiasis. It is believed that the benefits of combination therapy will be exploited as the shortcomings of each of the agents would have been overcome.

MATERIALS AND METHODS

Malaria parasite

Plasmodium berghei was used for the study. The strain used was obtained from the National Institute of Medical Research (NIMR), Lagos, Nigeria. The parasite was maintained in the animal house of the Department of Pharmacology and Toxicology, University of Uyo by serial passage of blood from one mouse to another intraperitoneally.

Drugs

Quinine and ciprofloxacin tablets used were obtained from a retail pharmacy outlet in Uyo, Nigeria.

Experimental Animals

Swiss albino mice (15-20g) of either sex were used for the study. They were obtained from the Animal house of the Faculty of Basic Medical Sciences, University of Uyo but were kept at the Animal house of the Department of Pharmacology and Toxicology of the University of Uyo where they were allowed free access to water and feed.

Innoculation of Experimental Animals

Each mouse was prescreened to confirm that they were not harbouring any malarial parasites and then inoculated with 0.2ml of infected blood (with *Plasmodium berghei*). After four days, the parasitaemia levels were determined in the animals before the administration of the drugs used for the study. In each case, the number of parasites was counted per 100 red blood cells.

Drug Administration

After inoculation with the infecting plasmodium, the infecting animals were divided into four groups of twelve mice per group. Each group was further divided into three sub groups with four mice each and they were treated with different doses of the drug or their combinations as shown in Table 1.

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Table1. Animal groupings, drug administration and doses

DRUGS	DRUG (mg/kg)	DOSAGE	GROUPS	SUBGROUPS	WEIGHT OF ANIMALS (g)
QUININE SULPHATE	4.3		1	A ₀₁	17 190
				A ₀₂	17 191
				A ₀₃	15 192
				A ₀₄	20 193
	8.6			B ₀₁	19 194
				B ₀₂	17 195
				B ₀₃	20 196
				B ₀₄	18 197
	12.9			C ₀₁	20 198
				C ₀₂	15 199
				C ₀₃	17 200
				C ₀₄	16 201
CIPROFLO XACIN	5.0		2	D ₀₁	19 202
				D ₀₂	20 203
				D ₀₃	17 204
				D ₀₄	15 205
	10.0			E ₀₁	16 206
				E ₀₂	15 207
				E ₀₃	15 208
				E ₀₄	20 209
	15.0			F ₀₁	19 210
				F ₀₂	15 211
				F ₀₃	15 212
				F ₀₄	20 213
QUININE SULPHATE + CIPROFLO XACIN	4.3 + 5.0		3	G ₀₁	16 214
				G ₀₂	20 215
				G ₀₃	17 216
				G ₀₄	15 217
	8.6+ 10.0			H ₀₁	15 218
				H ₀₂	15 219
				H ₀₃	20 220
				H ₀₄	19 221
	12.9 + 15.0			I ₀₁	18 222
				I ₀₂	18 223
				I ₀₃	20 224
				I ₀₄	19 225
CONTROL (DISTILLED WATER)	0		4	J ₀₁	15 226
				J ₀₂	20 227
				J ₀₃	15 228
				J ₀₄	16 229

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233 Preparation of drug solutions:
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235 **Quinine**
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237 One tablet of quinine (300mg) was powdered using mortar and pestle. The powder so
238 obtained was dissolved in 10mls of distilled water to achieve a concentration of
239 30mg/ml from which further concentrations which would be tolerated by the animals
240 were made. Note was taken of the fact that 121mg of quinine sulphate is equivalent to
241 100mg of quinine base.
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243 **Ciprofloxacin**
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245 One tablet of ciprofloxacin (500mg) was crushed and then powdered using mortar and
246 pestle. The powder obtained was obtained in 10mls of distilled water to obtain a
247 concentration of 50mg/ml. from this, further concentration were made as required.
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250 Preparation of Microscope slides
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252 Microscope slides were prepared using the method described by Fernando et al,(2008),
253 blood smears collected from freshly cut tails of the experimental animal were prepared
254 on a microscope slide, fixed with 3 drops of methanol for 5 minutes and air dried. The
255 air dried smear was then stained with Giemsa stain for 5 seconds, flushed with
256 distilled water and air dried. The slide was then viewed under the microscope(x100)
257 and the number of parasites per 100RBC counted.
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RESULTS

After four days of incubation of the parasites in the mice, signs of parasitaemia were observed viz-increase in body temperature, shivering and anaemia characterized by paleness. There was observed reduction in activities including movement of the whiskers. After the drug treatment with various dosages of the combination, signs of recovery were observed viz-fall in body temperature, improvement in the feeding and general improvement in the activity of the animal

The group of animals which served as control showed no signs of recovery from the burden of parasitaemia. The body temperature increased up to 40°C and the reduced movement of the limbs and whiskers were continuously observed. Eventually, all the animals died as the parasitaemia level rose to about 95 parasites per 100 red blood cells. (Table 4)

Table 2 shows results of the effect of quinine, ciprofloxacin and their combinations on mice infected with *P. berghei* while table 3 shows the daily decreases in parasitaemia level of mice treated with the same drugs

Table 2. Effect of treatment with different doses of quinine, ciprofloxacin and their combinations on mice infected with *P. berghei*

Drug dosage (mg/kg)	Parasitaemia level before drug treatment(Per 100Rbc)	Parasitaemia level after drug treatment (Per 100 Rbc)	Percentage reduction (%)
(4.3) {5.0} [4.3+5.0]	(72) {72} [71]	(43) {69} [36]	(40) {4} [49]
(8.6) {10.0} [8.6+10.0]	(71) {72} [73]	(24) {66} [16]	(66) {8} [78]
(12.9) {15.0} [12.9+15.0]	(73) {73} [73]	(18) {32} [9]	(75) {56} [87]

Key:

() = Results showing effect of different doses of quinine

{ } = Results showing effect of different doses of ciprofloxacin

[] = Results showing effect of different doses of combinations of quinine and ciprofloxacin

Table 3. Daily decreases in the parasitemia level of mice treated with quinine, ciprofloxacin and their combinations

DOSE (mg/kg)	Parasitaemia level (per 100 Rbc)			
	DAY 0	DAY 1	DAY 2	DAY 3
(4.3) { 5.0} [4.3+5.0]	(72) {72} [71]	(68) {72} [61]	(61) { 70} [49]	(43) {69} [36]
(8.6) {10.0} [8.6+10.0]	(71) { 72} [73]	(61) {71} [54]	(44) {70} [37]	(24) {66} [16]
(12.9) {15.0} [12.9+15.0]	(73) { 73} [73]	(46) {66} [50]	(28) {51} [28]	(18) {32} [9]

Key:

() = Results showing daily decreases in mean parasitaemia levels of different doses of quinine

{ } = Results showing daily decreases in mean parasitemia levels of different doses of ciprofloxacin

[] = Results showing daily decreases in mean parasitaemia levels of different doses of quinine and ciprofloxacin combination

Table 4. Daily increases in the parasitemia level of the control group

DOSE (mg/kg)	Parasitaemia level (per 100 Rbc)			
	DAY 0	DAY 1	DAY 2	DAY 3
0	73	80	89	90
0	72	79	88	90
0	74	81	90	95

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DISCUSSION

332 The burden of malaria remains heavy due to the development of multi drug resistance
333 (7). Hence malaria remains one of the most important diseases in the world and a
334 leading cause of death in children in Africa. Countries continue to deploy strategies to
335 control both the vector and the emergence of resistance against the control agents. One
336 of such strategies is the use of combination therapy.

337 The use of agents which have different molecular targets in combinations for therapy,
338 is known to be advantageous over their single use in monotherapy. This is because
339 they are known to accelerate therapeutic response, protect the component drugs
340 against resistance as well as shorten duration of use and hence improve compliance.
341 These benefits have intensified the search for effective antimalarials that can be used
342 in combinations.

343 The combinations used in this study demonstrated a significant reduction in
344 parasitaemia and enhanced the survival rate of the animals in the group when
345 compared with reduction in parasitaemia in mice treated with quinine or ciprofloxacin
346 alone

347 The parasitaemia level for the group of mice treated with the combination 2.9mg/kg of
348 quinine and 15mg/kg of ciprofloxacin showed the most significant decrease in
349 parasitaemia level from 73 parasites per 100RBC before treatment to 9 parasites per
350 100RBC after treatment when compared to decrease in parasitaemia level for single
351 treatment with 12.9mg/kg of quinine (From 73 parasites to 18 parasites per 100RBC)
352 and 15mg/kg of ciprofloxacin (From 73 parasites to 32 parasites per 100RBC).
353 However, combinations using lower doses of quinine and ciprofloxacin were not
354 useful as they could not reduce the parasitaemia reasonably. This is consistent with the
355 work of Salmon and his colleagues (8) and confirms that the effectiveness of this
356 combination is dose dependant. It is also consistent with the work of Andrade et al,
357 2007 who reported that the combination of Mefloquine or Artesunic acid with
358 ciprofloxacin is experimentally active against *P. falciparum*

359 The exact mechanism by which these agents work in combination was not studied
360 neither is the mechanism by which fluoroquinolones act against Plasmodium species
361 known. In bacteria however, they are known to inhibit DNA gyrase (9).

362 Conclusion

363 Result obtained in this study has shown that fluoroquinolones may be valuable
364 compounds in the treatment of plasmodial infections when used in combination with
365 rapidly active antimalarial agents. It has also shown that improved effect in malaria
366 treatment can be obtained when quinine is combined with ciprofloxacin.

We are interested in further pharmacokinetic studies between quinine and ciprofloxacin since their interaction could hamper the clinical use of the combination. We are also further interested in ascertaining the clinical implications of the increased dose of ciprofloxacin used.

It is believed that the result obtained will serve as a baseline data on the management of plasmodial infection using quinine and ciprofloxacin.

Conflict of Interest and ethical studies.

The authors declare that there is no conflict of interest regarding the publication of this article and that the animal studies were undertaken after prior approval of our institutions ethical committee

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