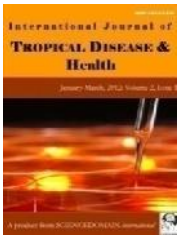

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# Effect of Combined Quinine and Ciprofloxacin Therapy in Experimental Murine Plasmodiasis

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Author MEUP designed the study and supervised the experiments. Author UCE co- supervised, wrote the first draft and managed the literature searches. Author OC managed the analyses of the study and also managed the literature searches. All authors read and approved the final manuscript.*

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## 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 anti malarial potentials. Effort in this study was therefore, devoted to evaluating in vivo, anti plasmodial activity of combination of varying doses (4.3, 8.6, 12.9 mg/kg body weight) of quinine (a known anti malarial gradually loosing 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 anti malarial 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.

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## 1. 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, anemia 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 anti malarial drugs involves the simultaneous use of two or even more anti malarial 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 anti malarial plus antibiotics provided that there is adequate evidence of their efficacy and safety [3].

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. [5], also confirms the efficacy of the combination of ciprofloxacin with an anti malarial (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 anti malarial but may have to be used at very high concentration to be able to achieve the required serum concentration for effectiveness [6]. 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 anti bacterial, especially those which are fast losing credibility as potent anti malarial 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 [7]. Always presented as a salt, quinine is known to have rapid schizonticidal action against intra erythrocytic malarial parasites. Quinine has been an anti malarial drug of choice over a long time even though, recently, it has been reported to lose its premium position to some other

anti malarial. This may not be unconnected with the challenge has been encountered using quinine, principal among which is poor tolerability and compliance with dosing regimen.

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.

## 2. MATERIALS AND METHODS

### 2.1 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.

### 2.2 Drugs

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

### 2.3 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.

### 2.4 Inoculation of Experimental Animals

Each mouse was prescreened to confirm that they were not habouring 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.

### 2.5 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.

### 2.6 Preparation of Drug Solutions

#### 2.6.1 Quinine

One tablet of quinine (300mg) was powdered using mortar and pestle. The powder so obtained was dissolved in 10ml of distilled water to achieve a concentration of 30mg/ml from which further concentrations which would be tolerated by the animals were made. Note was taken of the fact that 121mg of quinine sulphate is equivalent to 100mg of quinine base.

### 2.6.2 Ciprofloxacin

One tablet of ciprofloxacin (500mg) was crushed and then powdered using mortar and pestle. The powder obtained was obtained in 10ml of distilled water to obtain a concentration of 50mg/ml from this, further concentration were made as required.

**Table 1. Animal groupings, drug administration and doses**

| Drugs | Drug dosage (mg/kg) | Groups | Subgroups | Weight of animals (g) |
|---|---|---|---|---|
| Quinine Sulphate | 4.3 | 1 | $A_{01}$ | 17 |
| | | | $A_{02}$ | 17 |
| | | | $A_{03}$ | 15 |
| | | | $A_{04}$ | 20 |
| | 8.6 | | $B_{01}$ | 19 |
| | | | $B_{02}$ | 17 |
| | | | $B_{03}$ | 20 |
| | | | $B_{04}$ | 18 |
| | 12.9 | | $C_{01}$ | 20 |
| | | | $C_{02}$ | 15 |
| | | | $C_{03}$ | 17 |
| | | | $C_{04}$ | 16 |
| Ciprofloxacin | 5.0 | 2 | $D_{01}$ | 19 |
| | | | $D_{02}$ | 20 |
| | | | $D_{03}$ | 17 |
| | | | $D_{04}$ | 15 |
| | 10.0 | | $E_{01}$ | 16 |
| | | | $E_{02}$ | 15 |
| | | | $E_{03}$ | 15 |
| | | | $E_{04}$ | 20 |
| | 15.0 | | $F_{01}$ | 19 |
| | | | $F_{02}$ | 15 |
| | | | $F_{03}$ | 15 |
| | | | $F_{04}$ | 20 |
| Quinine Sulphate+ Ciprofloxacin | 4.3 + 5.0 | 3 | $G_{01}$ | 16 |
| | | | $G_{02}$ | 20 |
| | | | $G_{03}$ | 17 |
| | | | $G_{04}$ | 15 |
| | 8.6+ 10.0 | | $H_{01}$ | 15 |
| | | | $H_{02}$ | 15 |
| | | | $H_{03}$ | 20 |
| | | | $H_{04}$ | 19 |
| | 12.9 + 15.0 | | $I_{01}$ | 18 |
| | | | $I_{02}$ | 18 |
| | | | $I_{03}$ | 20 |
| | | | $I_{04}$ | 19 |
| Control (Distilled water) | 0 | 4 | $J_{01}$ | 15 |
| | | | $J_{02}$ | 20 |
| | | | $J_{03}$ | 15 |
| | | | $J_{04}$ | 16 |

## 2.7 Preparation of Microscope Slides

Blood smears collected from freshly cut tails of the experimental animal were prepared on a microscope slide, fixed with 3 drops of methanol for 5minutes and air dried. The air dried smear was then stained with Giemsa stain for 5 seconds, flushed with distilled water and air dried. The slide was then viewed under the microscope(x100) and the number of parasites per 100RBC counted.

## 3. RESULTS

After four days of incubation of the parasites in the mice, signs of parasitaemia were observed viz-increase in body temperature, shivering and anemia 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$^{o}$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 2)

Table 3 shows results of the effect of quinine, ciprofloxacin and their combinations on mice infected with *P. berghei* while Table 4 shows the daily decreases in parasitaemia level of mice treated with the same drugs.

**Table 2. Daily increases in the Parasitaemia 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 |

**Table 3. 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 4. Daily decreases in the Parasitaemia 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 Parasitaemia levels of different doses of ciprofloxacin*
*[ ] = Results showing daily decreases in mean parasitaemia levels of different doses of quinine and ciprofloxacin combination.*

## 4. DISCUSSION

The burden of malaria remains heavy due to the development of multi drug resistance [8]. Hence malaria remains one of the most important diseases in the world and a leading cause of death in children in Africa. Countries continue to deploy strategies to control both the vector and the emergence of resistance against the control agents. One of such strategies is the use of combination therapy.

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

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

The parasitaemia level for the group of mice treated with the combination 2.9mg/kg of quinine and 15mg/kg of ciprofloxacin showed the most significant decrease in parasiataemia level from 73 parasites per 100RBC before treatment to 9 parasites per 100RBC after treatment when compared to decrease in parasitaemia level for single treatment with 12.9mg/kg of quinine (From 73 parasites to18 parasites per 100RBC) and 15mg/kg of ciprofloxacin (From 73 parasites to 32 parasites per 100RBC). However, combinations using lower doses of quinine and ciprofloxacin were not useful as they could not reduce the parasitaemia reasonably. This is consistent with the work of Salmon and his colleagues [9] and confirms that the effectiveness of this combination is dose dependent. It is also consistent with the work of Andrade et al, 2007 who reported that the combination of Mefloquine or Artesunic acid with ciprofloxacin is experimentally active against P. *falciparum*

The exact mechanism by which these agents work in combination was not studied neither is the mechanism by which floroquinolones act against Plasmodium species known. In bacteria however, they are known to inhibit DNA gyrase [10].

## 5. CONCLUSION

Result obtained in this study has shown that floroquinolones may be valuable compounds in the treatment of plasmodial infections when used in combination with rapidly active anti

malarial agents. It has also shown that improved effect in malaria 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.

## CONSENT

Not applicable.

## ETHICAL APPROVAL

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.

## COMPETING INTEREST

Authors have declared that no competing interests exist.

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