# Amodiaquine–Ciprofloxacin: a potential combination therapy against drug resistant malaria

Y. F. FALAJIKI[1,2], O. AKINOLA[1,2], O. O. ABIODUN[1,2], C. T. HAPPI[2,3], A. SOWUNMI[1,2] *and* G. O. GBOTOSHO[1,2]*

[1] *Department of Pharmacology and Therapeutics, College of Medicine, University of Ibadan, Ibadan, Nigeria*
[2] *Malaria Research Laboratories, Institute for Advanced Medical Research and Training, College of Medicine, University of Ibadan, Ibadan, Nigeria*
[3] *Department of Biological Sciences, Redeemer University of Nigeria, Mowe, Ogun state, Nigeria*

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SUMMARY

Emergence of malaria parasites resistant to artemisinin necessitates the need for development of new antimalarial therapies. Ciprofloxacin (CFX) a second generation quinolone antibiotic possesses some antimalarial activities. We investigated the *in vivo* antimalarial activities of CFX in combination with amodiaquine in mice infected with chloroquine-resistant *Plasmodium berghei* ANKA. Animals were treated orally with 80 or 160 mg kg$^{-1}$ body weight of CFX alone given twice daily or in combination with amodiaquine (AQ) 10 mg kg$^{-1}$ body weight. Parasitological activity and survival of the animals were assessed over 21 days. Peak parasitaemia in the untreated control group was 72·51%. Treatment with AQ alone resulted in clearance of parasitaemia by day 4 while treatment with CFX 80 and 160 mg kg$^{-1}$ alone suppressed parasitaemia by 13·94–54·64% and 35·6–92·7%, respectively. However, the combination of CFX with AQ significantly enhanced response of infection in the animals to treatment ($P < 0·05$) resulting in complete resolution of parasitaemia throughout follow up period with CFX 160 mg kg$^{-1}$, delayed recrudescence time with CFX 80 mg kg$^{-1}$ and significant increase in survival rate of the animals. The results demonstrate beneficial interaction between AQ and CFX which may provide a clinically relevant antimalarial/antibiotic therapeutic option in the management of malaria.

Key words: Malaria, *Plasmodium berghei*, amodiaquine-ciprofloxacin, drug combinations.

## INTRODUCTION

Malaria is one of the world's most difficult health problems to solve, with several hundred million debilitating cases of the disease yearly, killing an estimated 660 000 people, mostly children under the age of five (WHO, 2012). Majority of these deaths occur in sub Saharan Africa where the disease is highly endemic and most difficult to manage. Efforts to control this disease are hampered by drug resistance in parasites, insecticide resistance in mosquitoes, and lack of an effective vaccine (Maciel *et al*. 2008). Initiatives by the World Health Organization (WHO), the Gates Foundation, the Global Fund and the World Bank for reduction and eradication of this disease are achieving great success (Co *et al*. 2010). This is attributable to a number of actions, most significant of which is the recommendation of artemisinin-based combination therapy (ACT) as first line treatment of uncomplicated malaria by the World Health Organization (WHO, 2010). Unfortunately, the success achieved so far is now being confounded by increasing reports of *Plasmodium falciparum* resistance to ACT (Phyo *et al*. 2012; WWARN, 2012; Rogers *et al*. 2009; Dondorp *et al*. 2009; Noedl *et al*. 2008). The emergence of parasites resistant to artemisinin, and the development of multidrug-resistant strains is now of great concern and thus, there is an urgent need for the development of therapies that can counter the spread of existing and emerging drug resistance. A strategy being considered in fighting the spread of plasmodium resistance is to reposition, repurpose or find new uses for drugs that are already used for other indications (Weisman *et al*. 2006; Nzila *et al*. 2011). This has been exploited in the past in the treatment of malaria and tuberculosis with the use of sulpha-based drug and fluoroquinolones, respectively, which were initially developed for other indications (Nzila *et al*. 2011). One class of drugs that has been found to possess antimalarial activity are the fluoroquinolone antibiotics. They possess several favourable properties such as excellent bioavailability, good tissue penetrability and a relatively low incidence of adverse and toxic effects (Sharma *et al*. 2009). Several studies have reported the potency of fluoroquinolones against blood and hepatic stages of *P. falciparum* isolates *in vitro* (Tripathi *et al*. 1993; Yeo *et al*. 1998; Hamzah *et al*. 2000; Pradines *et al*. 2001; Mahmoudi *et al*. 2003; Dahl and Rosenthal, 2007). Ciprofloxacin (CFX), norfloxacin and Pefloxacin have been clinically assessed in patients with acute uncomplicated *P. falciparum* malaria and results from the studies indicate the

* Correspondence author. Department of Pharmacology and Therapeutics, College of Medicine, University of Ibadan, Ibadan, Nigeria. E-mail: solagbotosho@yahoo.co.uk

 
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potential of fluoroquinolones as possible antimalarials (Sarma 1989; Deloron *et al*. 1991; McClean *et al*. 1992; Stromberg and Bjorkman 1992). However, in some of these trials, there were limitations to the potency of the drugs in achieving sufficient efficacy against falciparum malaria. This was attributed to a delayed effect of the drugs on the parasites and probably the low dose of the drugs administered during the trials. These drawbacks limit the potential clinical usefulness of fluoroquinolones as antimalarials but they have been proposed to be considered for use in combination with rapidly acting antimalarial drugs (Pradines *et al*. 2001). The potential clinical value of CFX as a partner drug with more rapidly acting antimalarial drugs including mefloquine and chloroquine against drug resistant plasmodium infection has been documented (Andrade *et al*. 2007; Gbotosho *et al*. 2012). However, pharmacokinetic interaction between chloroquine and CFX in the studies appeared to limit the efficacy of the combination of lower doses of CFX with chloroquine (Ilo *et al*. 2006, 2008; Gbotosho *et al*. 2012). This represents a major drawback and necessitates the need to evaluate the combination of other rapidly acting antimalarial drugs with CFX. In the present study, the interaction between CFX and amodiaquine (AQ) was evaluated in a murine model to determine the potential benefits of the combination in the treatment of chloroquine-resistant *Plasmodium berghei* infection.

## MATERIALS AND METHOD

### *Drug samples*

AQ was obtained from Sigma, St. Louis, MO, USA and CFX was graciously provided by Bond Pharmaceuticals, Awe, Oyo State, Nigeria. Stock solutions of the compounds were prepared in distilled water and stored at −20 °C till time of usage.

### *Animals*

Experimental animals used in this study were male Swiss albino mice (7–10 weeks old) weighing 20–22 g. The animals were obtained from the animal facility of the Malaria Research Laboratories, Institute of Advanced Medical Research and Training (IMRAT), University of Ibadan, Ibadan. The mice were used in accordance with the NIH Guide for the care and use of laboratory animals, NIH publication (volume 25, number 28), revised 1996.

### *Antimalarial test* in vivo

A modification of the Peter's suppressive tests *in vivo* (Peters, 1965) was used. Briefly, 35 male albino mice weighing 20–22 g were inoculated intravenously through the tail vein with $1 \times 10^6$ red blood cells infected with CQ-resistant *P. berghei* ANKA strain. The infected animals were separated into 7 groups of 5 mice per group and were treated by oral drug administration 2 h post infection. Groups of animals receiving varying doses of CFX alone (40, 80 or 160 mg $kg^{-1}$ body weight) or CFX (80 and 160 mg $kg^{-1}$) in combination with AQ were treated twice daily for 5 days. AQ (10 mg $kg^{-1}$ body weight) was administered once daily for 3 days. Two control groups were used, one treated with AQ alone at 10 mg $kg^{-1}$ body weight given daily for 3 days while the second control group were treated with normal saline. Drugs stocks were diluted to the desired final concentration with distilled water so that each animal received 200 $\mu$l at time of administration of each drug.

Parasiticidal activity was assessed daily from day 4 post infection till day 14, and then on days 21, 28, 35 and 42. Blood smears were prepared from the tail, methanol-fixed, stained with Giemsa and microscopically examined by counting parasitaemia in 1000 erythrocytes. Mortality was monitored daily. Inhibition of parasite growth in drug treated group was calculated in relation to parasite growth in the non-drug treated control group. All treatment regimens were tested in three independent experiments.

### *Statistical analysis*

Student's *t*-test was used to analyse the differences in mean parasitaemia level on days following treatment initiation, and analysis of variance between groups (ANOVA) was used to compare difference in percentage inhibition of parasite growth. Chi-square with Yates correlation was used in analysing the proportions of survival.

## RESULTS

The mean percentage parasitaemia versus time in the untreated control animals and in animals treated with CFX or AQ alone are shown in Fig. 1. The mean percentage parasitaemia in the untreated control group ranged from 1·8% on day 4 post infection to 72·51% on day 14 when it peaked. By day 16 post infection, all animals in the control group had died. Animals treated with AQ (10 mg $kg^{-1}$) alone, had 100% suppression of parasitaemia ($P < 0{\cdot}05$) till day 9 post infection after which recrudescence of parasitaemia was observed on day 10. In the group of animals treated with CFX (40 mg $kg^{-1}$) alone there was no significant difference ($P > 0{\cdot}05$) in parasite suppression when compared to the untreated control as both groups showed a similar parasite growth profile. In contrast, parasite suppression in the group of animals that received 80 mg $kg^{-1}$ body weight of CFX alone ranged from 13·94 to 54·64% relative to the untreated control with the highest suppression occurring on day 10

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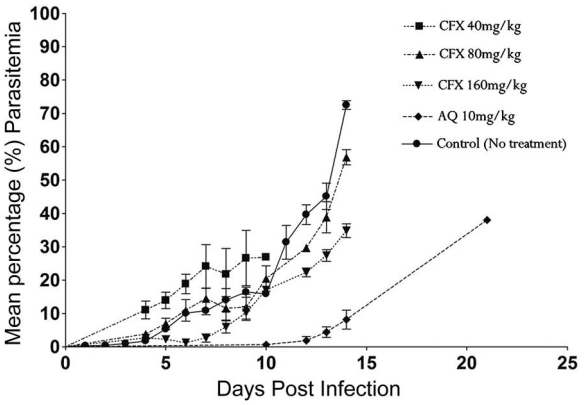


Fig. 1. Response of chloroquine-resistant *P. berghei* ANKA infection in mice to treatment with AQ alone and varying doses of CFX.

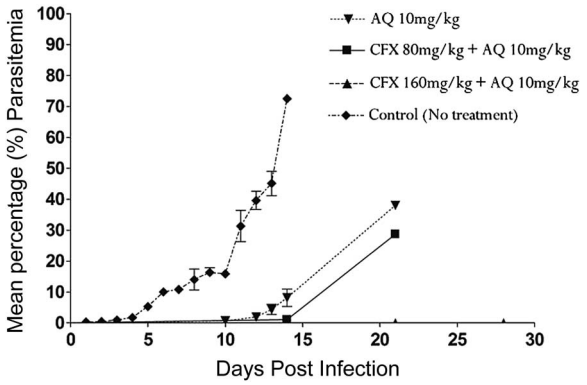


Fig. 2. Comparative response of chloroquine-resistant *P. berghei* ANKA infection to AQ alone, or AQ in combination with 80 mg $kg^{-1}$ or 160 mg $kg^{-1}$ CFX.

post infection. In the group of animals that received 160 mg $kg^{-1}$ of CFX alone, there was a significant reduction in parasitaemia ranging from 35·6 to 92·7% during the follow up period ($P < 0·05$). The highest suppression occurred on day 6.

### *Assessment of antimalarial activity of AQ alone and AQ plus CFX*

In the group of animals that received AQ combined with 80 mg $kg^{-1}$ CFX [AQ+CFX (80)], the parasite clearance time was similar to the group of animals that received AQ alone, however mean parasite recrudescence time was significantly longer in the AQ+CFX (80) group compared to the group of animals that received AQ (10 mg $kg^{-1}$) alone ($P = 0·018$). Recrudescence occurred in the AQ alone treatment group on day 10 (mean parasitaemia on day 10 = 0·16%) while treatment with AQ+CFX (80) showed 100% suppression of parasitaemia till day 13 post infection with parasite recrudescence occurring on day 14 (mean parasitaemia on day 14 = 0·28% versus 8·18% in the AQ only treatment group on day 14) (Fig. 2). The combination of AQ 10 mg $kg^{-1}$ and CFX 160 mg $kg^{-1}$ [AQ+CFX (160)] resulted in complete parasite suppression without recrudescence in any of the animals throughout the period of follow up (Fig. 2). Results of the parasite clearance and recrudescence time in the different treatment groups are shown on Table 1. The mean parasitaemia in the different treatment groups after recrudescence are shown in Table 2. Parasitaemia after recrudescence in the animals was 4–29 folds lower in the AQ+CFX 80 mg $kg^{-1}$ compared to the AQ alone treatment group between days 14 and 21.

### *Survival rate of animals*

The rate of survival of animals treated with AQ alone, CFX alone or AQ in combination with varying doses of CFX on day 21 post infection is shown in Table 3. The day-21 survival rate in animals treated with CFX (40, 80 and 160 mg $kg^{-1}$) alone was 0% as all animals died between day 10 and 18. The day 21 survival rates in the group of animals that received AQ alone or AQ combine CFX 80 mg $kg^{-1}$ or CFX 160 mg $kg^{-1}$ were 40, 100 and 80%, respectively. There was significant difference between the survival rates of the animals in the AQ only treatment group compared with the group of animals that received AQ combined with CFX ($P = 0·0035$). The only animal that died in the AQ+CFX 160 mg $kg^{-1}$ group died on day 18, despite the fact that parasitaemia was completely suppressed in animals in this treatment group. The death recorded in this animal may have been from toxicity based on observed status (weight loss, sluggish movement and loss of fur) of the mouse before its eventual death.

## DISCUSSION

Reports of reduced *in vivo* and *in vitro* susceptibilities of *P. falciparum* to artemisinin derivatives in western Cambodia, Thailand, French Guiana, Senegal and Nigeria (Jambou *et al*. 2005; Dondorp *et al*. 2009; Rogers *et al*. 2009; Bustamante *et al*. 2012; Phyo *et al*. 2012), highlight the urgent need to develop new and alternative antimalarial drugs. The fluoroquinolones are synthetic antibiotics with broad spectrum of activity against gram-positive and gram-negative bacteria and mycobacterium (Da Silva *et al*. 2003). The antimalarial activity of fluoroquinolones including CFX against *P. berghei in vivo* and chloroquine sensitive and resistant strains of *P. falciparum in vitro* has been well documented (Salmon *et al*. 1990; Mahmoudi *et al*. 2003). More interestingly, although limited, studies *in vitro* and *in vivo* in mice have described beneficial interaction between CFX and chloroquine or mefloquine against *P. falciparum and P. berghei* infections (Kazzim *et al*. 2006; Andrade *et al*. 2007; Gbotosho *et al*. 2012) thus providing a potential

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Table 1. Parasite clearance and recrudescence time after treatment with AQ alone, or selected doses of CFX (alone), or combination of AQ with selected doses of CFX in mice infected with chloroquine-resistant *P. berghei* ANKA

| Treatment groups | Parasite clearance time (days post infection) | Recrudescence time (days post infection) |
|---|---|---|
| AQ (10 mg $kg^{-1}$) | 1·00 ± 0·00 | 10·00 ± 0·00 |
| AQ (10 mg $kg^{-1}$) + CFX (80 mg $kg^{-1}$) | 1·00 ± 0·00 | 14·00 ± 0·00 |
| AQ (10 mg $kg^{-1}$) + CFX (160 mg $kg^{-1}$) | 1·00 ± 0·00 | NPR |
| CFX (160 mg $kg^{-1}$) | NPC | – |
| CFX (80 mg $kg^{-1}$) | NPC | – |
| CFX (40 mg $kg^{-1}$) | NPC | – |

NPC - NO PARASITE CLEARANCE; NPR - NO PARASITE RECRUDESCENCE.

Table 2. Mean percentage parasitaemia after parasite recrudescence in animals infected with chloroquine-resistant *P. berghei* and treated with AQ alone or AQ combined with varying doses of CFX

| Days post infection | AQ (10 mg $kg^{-1}$) | AQ+CFX (80 mg $kg^{-1}$) | AQ+CFX (160 mg $kg^{-1}$) |
|---|---|---|---|
| 10 | 0·16 ± 0·16 | 0·00 ± 0·00 | 0·00 ± 0·00 |
| 12 | 1·90 ± 1·22 | 0·00 ± 0·00 | 0·00 ± 0·00 |
| 13 | 4·42 ± 1·61 | 0·00 ± 0·00 | 0·00 ± 0·00 |
| 14 | 8·18 ± 2·82 | 0·28 ± 0·28 | 0·00 ± 0·00 |
| 21 | 36·52 ± 2·86 | 9·34 ± 1·75 | 0·00 ± 0·00 |
| 28 | –[a] | 21·6 ± 3·80 | 0·00 ± 0·00 |

[a] All dead.

Table 3. The survival rate of animals infected with chloroquine resistant *P. berghei* ANKA and treated with varying doses of CFX alone, AQ alone or AQ combined with varying doses of CFX

| Treatment | Response Percent survival day 21 |
|---|---|
| CFX 40 mg $kg^{-1}$ | 0 |
| CFX 80 mg $kg^{-1}$ | 0 |
| CFX 160 mg $kg^{-1}$ | 0 |
| AQ 10 mg $kg^{-1}$ | 40 |
| AQ 10 mg $kg^{-1}$ + CFX 80 mg $kg^{-1}$ | 100 |
| AQ 10 mg $kg^{-1}$ + CFX 160 mg $kg^{-1}$ | 80 |
| Control (no treatment) | 0 |

alternative chemotherapeutic strategy. Unfortunately, pharmacokinetic interactions between chloroquine and CFX appeared to hinder efficacy of the combination in mice infected with *P. berghei* especially at lower doses of CFX (Ilo *et al*. 2008; Gbotosho *et al*. 2012) thus limiting the potential clinical use of the chloroquine-CFX combination. Our study describes a dose-dependent potentiation of intrinsic antimalarial activity of AQ against chloroquine-resistant *P. berghei* by CFX. The efficacy of the combination was demonstrated by enhanced sensitivity of *P. berghei* infection in mice to the combination of AQ plus CFX and enhanced survival rates of the animals compared with response to AQ or CFX alone. CFX, at the two doses employed (80 and 160 mg $kg^{-1}$) in this study had a significantly greater beneficial effect on AQ activity against *P. berghei* compared to its effect on chloroquine activity against the parasites in previous studies (Gbotosho *et al*. 2012). This is the first report of interaction of CFX with AQ raising hope of potential clinical application of this antibiotic/antimalarial combination. The combination of AQ combined with CFX appears to provide a more superior clinically relevant antimalarial/antibiotic therapeutic option in the management of malaria than the chloroquine with CFX combination. The highest dose of CFX employed in this study produced complete clearance of parasitaemia in the animals without any recrudescence throughout the duration of the study when combined with AQ. In the study by Gbotosho *et al*. (2012) beneficial interaction was only recorded in the combination of CFX (160 mg $kg^{-1}$) with chloroquine which achieved a significant reduction in parasitaemia without complete parasite clearance. The superiority of the AQ/CFX combination is

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further highlighted with the intermediate dose of CFX (80 mg $kg^{-1}$) achieving maximum suppression up till day 13 post infection. In addition, parasitaemia in the animals that received AQ+CFX 80 mg $kg^{-1}$ was significantly lower (4–29 folds) than in animals that received AQ alone during recrudescence of parasitaemia. The superiority of the AQ/CFX combination was also demonstrated in the day-21 survival rates of the animals. The group of animals that received AQ+CFX 80 mg $kg^{-1}$ exhibited 100% survival while no animal survived by day 21 after receiving CQ+CFX 80 mg $kg^{-1}$ in a previously reported study (Gbotosho *et al*. 2012).

Pharmacokinetic interaction between CFX and chloroquine has been reported (Ilo *et al*. 2006, 2008) leading to increased rate of CFX excretion and decreased bioavailability of CFX which appears to have hindered the expected beneficial effect between CFX and chloroquine especially at lower doses of CFX (Gbotosho *et al*. 2012). Although AQ and chloroquine are both 4-aminoquinolines, results from this study indicates that the nature of interaction between CFX and AQ appears to differ from its interaction with chloroquine. AQ is a known antimalarial drug and there are documented reports of antimalarial activity of CFX, *in vitro* and *in vivo* in animal models. However, detailed studies are required to establish the efficacy of the combination against malaria, while pharmacokinetic studies are required to understand the nature of interaction and determine appropriate doses prior to clinical application of the combination.

Antibiotic/antimalarial combinations provide a potentially useful alternative chemotherapeutic strategy in control of malaria in disease endemic regions. Clarithromycin, a macrolide antibiotic and potent inhibitor of cytochrome $P_{450}$ 3A4 has been shown to significantly reverse mefloquine resistance in *Plasmodium yoelii nigeriensis* (Tripathi *et al*. 2011). In that study, the combination resulted in 100% cure rate. Similarly, synergistic interaction between clarithromycin and quinine or quinidine *in vitro* against *P. falciparum* and *in vivo* against *P. yoelii nigeriensis* has been documented (Pandey *et al*. 2013). It is postulated that clarithromycin might have caused reduced CYP3A4 activity leading to increased plasma level of quinine and quinidine to produce enhanced antimalarial activity. Furthermore, parasite apicoplast disruption by clarithromycin synergizes the antimalarial action of quinine and quinidine. Although the nature of interaction between AQ and CFX in our study is not clearly defined, our results are consistent with a previous report of synergistic interaction between mefloquine and lower doses of CFX in mice infected with chloroquine-sensitive *P. berghei* (NK 65) as well as *in vitro* against *P. falciparum* (Andrade *et al*. 2007). The findings in our study and other reports thus represent a potentially good starting point for development of new drug combinations.

In our study, evidence of toxicity was observed in the form of sluggishness, weight loss and loss of fur in some animals treated with AQ in combination with CFX 160 mg $kg^{-1}$ although, there was no patent parasitaemia in this group of animals. This finding was consistent with reports from previous studies with the combination of chloroquine and CFX 160 mg $kg^{-1}$ (Gbotosho *et al*. 2012). The doses of CFX employed in this study were selected based on prior studies on activities of CFX in mice (Salmon *et al*. 1990; Gbotosho *et al*. 2012). In the study by Salmon *et al* (1990), doses of CFX ranging from 40 mg $kg^{-1}$ body weight to 240 mg $kg^{-1}$ body weight were employed with the lower dosages of 40 and 80 mg $kg^{-1}$ body weight being poorly active against the chloroquine resistant N67 strain of *Plasmodium yoelii*. Although the doses of CFX employed in the present study probably give concentrations in blood higher than those clinically achievable in humans, the therapeutic activities achieved suggest that the combination of CFX and standard dose of AQ might prove useful in the treatment of malaria.

The potential target of CFX in malaria parasites is suggested to be the apicoplast with the mechanism of action related to the dysfunction of this organelle (Dahl and Rosenthal, 2007), thus, it presents an opportunity to selectively combat malaria parasites with little adverse effect on the host, as the apicoplast is known to be a viable target within parasites. The potentiation of antimalarial activity of AQ by CFX observed in this study raises the hope for developing antibiotic/antimalarial combinations. However, detailed pharmacokinetic, toxicological and dose finding studies are required to further validate the beneficial interaction of the combination prior to clinical application of the combination.

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