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Antimalarial Activity of Amodiaquine-Moxifloxacin: A Study in Mice

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Abstract

The search for new partner drugs to increase the therapeutic activity of existing antimalarial drugs is important because of decreased Plasmodium susceptibility. Amodiaquine (AQ) is an antimalarial drug. Moxifloxacin (MX) is a fluoroquinolone antibiotic with promising antiplasmodial activity. This study evaluated the benefit of MX as a partner drug with AQ for malaria treatment in *Plasmodium berghei* infected mice. Adult Swiss albino mice (28 g to 35 g) of both genders, randomly grouped and inoculated with *Plasmodium berghei* were used. The mice were treated orally with AQ (10 mg/kg), MX (6 mg/kg) and AQ-MX, respectively using the curative, prophylactic and suppressive protocols. Blood samples were collected and assessed for percentage parasitemia and hematological indices. Liver samples were assessed for histological changes. Mean Survival Time (MST) was observed in treated mice. The curative, prophylactic and suppressive tests showed that AQ-MX decreased percentage parasitemia with difference observed at $p<0.05$ when compared to AQ or MX. In the curative test, AQ, MX and AQ-MX produced 70.9%, 65.0% and 90.6% parasitemia inhibitions, respectively whereas CQ (Standard) produced 87.9% parasitemia inhibition. AQ-MX prolonged MST with difference observed at $p<0.05$ in the curative, prophylactic and suppressive tests when compared to AQ or MX. The restored hematological indices caused by AQ-MX were characterized by increased hemoglobin, red blood cells and packed cell volume with decreased white blood cells observed at $p<0.05$ when compared to AQ or MX. AQ-MX eradicates liver *Plasmodium berghei*. MX may be an effective partner drug with AQ for malaria treatment.

Keywords: Amodiaquine; Moxifloxacin; Partner-drug; Plasmodium; Mice

Introduction

Malaria persists in tropical and sub-tropical regions of the world despite concerted global effort that dates back to the World Health Organization (WHO) global malaria eradication programmer in 1950's and 1960's [1]. The underprivileged, rural populations consisting of young children and pregnant women are disproportionately affected by malaria. The cornerstone of malaria control efforts for the past decades has been to provide antimalarial commodities toward the prevention and eradication of malaria [2].

One of the challenges in the treatment of malaria especially in the tropics is the emergence of resistant to most antimalarial drugs. Combination therapy especially Artemisinin Combination Therapies (ACTs), which combines artemisinins with partner drugs were introduced to overcome the incidence of resistance. ACTs have produced remarkable success against Plasmodium resistance however; there is gradual emergence of Plasmodium resistance to ACTs. The de novo emergence of resistance can be prevented by the continual exploration of new antimalarial drug combinations. New combinations can delay or slow emergence and spread of resistance by eliminating resistant mutants except those that carry two different mutations [3].

Amodiaquine (AQ) is an orally active 4 aminoquinoline derivative with antimalarial and anti-inflammatory properties similar to chloroquine. It is used for the treatment of malaria including uncomplicated Plasmodium falciparum malaria. AQ has been used with good outcomes as a partner drug with artesunate, sulfadoxine and pyrimethamine to reduce the risk of resistance [4]. Moxifloxacin (MX) belongs to the fluroquinoline family, it is a broad spectrum antibiotics, which is active against gram positive and gram negative bacteria. In bacteria, it inhibits DNA gyrase and topoisomerase IV. MX has been associated with antiplasmodial activity against *P. falciparum* strains with the suggestion that it may serve as a viable partner drug to artemisinins and other antimalarial drugs. This study assessed whether MX could be a viable partner drug with AQ in *P. berghei*-infected mice [5].

Materials and Methods

Animals, drugs and parasites

Swiss albino mice of both genders (28 g to 35 g) used for this study were sourced from the animal husbandry of the department of pharmacology, faculty of basic clinical sciences, university of port harcourt, Rivers state [6]. The mice were housed in cages and acclimated for 2 weeks with access to food pellets and water freely. The mice were handled according to the guide on animal handling by European council and the Parliament. Doses were selected based on previous studies: CQ (10 mg/kg), MX (2.2 mg/kg) [17] and AQ (1.71/13.7 mg/kg). Donor mice infected with CQ-sensitive strain of *P. berghei* (NK65) used were obtained from Nigerian institute of medical research, Yaba, Lagos [7].

Parasite inoculation

Stock inoculation containing 1 × 107 *P. berghei* infected erythrocytes in 0.2 ml was prepared by diluting portion of the blood infected with *P. berghei* with 0.9% normal saline. Erythrocytes containing 1 × 107 *P. berghei* was inoculated into each mouse through intraperitoneal (ip) route [8].

Determination of antiplasmodial curative activity

The method described by Ryley and Peters (1970) was used. Twenty-five mice grouped into 5 of 5 mice/group were used. The mice were parasitized with 1 × 107 *P. berghei* (i.p) except for the negative control. After 3 days, the mice were treated per oral (p.o) with AQ (10 mg/kg), MX (5 mg/kg) and AQ-MX daily for 4 days. The parasitized control was treated with normal saline (0.2 mL), whereas the positive (standard) control was treated with CQ (10 mg/kg) daily for 4 days. On day 8, blood samples were collected from the tail and thin blood films were produced on microscope slides. The films were fixed with 10% giemsa stain for 30 min and examined under oil immersion × 100 magnification [9]. The number of parasitized Red Blood Cells (RBCs) were counted against the total number of RBCs in a field. Percentage parasitemia levels were calculated as shown below.

$$\%\ \text{Parasitemia} = \frac{\text{Number of parasitized red blood cells (RBCs)}}{\text{Total number of RBCs count}} \times 100\%$$

$$\%\ \text{Inhibition} = \frac{(\%\ \text{Parasitemia of negative control} - \%\ \text{Parasitemia of treated group})}{\%\ \text{Parasitemia of negative control}}$$

Determination of antiplasmodial suppressive activity

The method described by Knight and Peters (1980) was used. Twenty five mice were randomly grouped into grouped into 5 of n=5/group and parasitized with 1 × 107 *P. berghei* ip for 3 h. Thereafter, the mice were treated per oral (p.o) with AQ (10 mg/kg), MX (6 mg/kg) and AQ-MX daily for 4 days. The parasitized control was treated with normal saline (0.2 mL), whereas the positive (standard) control was treated with CQ (10 mg/kg) daily for 4 days [10]. On day 5, blood samples were collected from the tail and thin films were prepared on slides. Percentage parasitemia levels were calculated as shown above [11].

Determination of antiplasmodial prophylactic activity

The method described by Peters (1975) was used for prophylactic test. Twenty five mice randomized into 5 groups n=5/group were used. The mice were treated per oral (p.o) with AQ (10 mg/kg), MX (6 mg/kg) and AQ-MX daily for 4 days. The parasitized control the positive (standard) control were treated with normal saline (0.2 mL) and CQ (10 mg/kg) daily for 4 days, respectively. On day 4, except for the negative control the mice were inoculated with 1 × 107 *P. berghei* ip and treatment continued for 4 days. Blood samples were collected from the tail and percentage parasitemia levels was determined as stated above [12].

Determination of Mean Survival Time (MST)

The mice in the control and the treated groups were observed for mortality and expressed in days. Mortality expressed Mean Survival Time (MST) was calculated as shown below [13].

$$\text{MST} = \frac{\text{Sum of survival time of all mice in a group (days)}}{\text{Total number of mice in that group}}$$

Evaluation of hematological and lipid parameters

Blood samples from the mice in the curative study were collected and assessed for Packed Cell Volume (PCV), Red Blood Cells (RBCs), Hemoglobin (HB), White Blood Cells (WBCs), High-Density Lipoprotein Cholesterol (HDL-C), Low-Density Lipoprotein Cholesterol (LDL-C) and Triglyceride (TG) concentrations using an auto analyzer [14].

Statistical analysis

Data are mean ± Standard Error of Mean (SEM). Differences between groups were determined using one-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test. Significance was considered at $P<0.05$ [15].

Results

Curative effect of amodiaquine-moxifloxacin on mice infected with *Plasmodium berghei*. Treatment with AQ-MX decreased percentage parasitemia significantly at $p<0.05$ when compared to individual doses of AQ and MX. AQ, MX and AQ-MX showed inhibitions of 65.62%, 62.03% and 85.31%, respectively, while CQ produced 83.72% inhibition [16]. Treatment with AQ-MX significantly prolonged MST when compared to AQ or MX with significance observed at $p<0.05$ (Table 1).

Treatment	% Parasitamia	% Inhibition	MST (Days)
PC	31.26 ± 1.23^{a}	0	9.05 ± 0.97^{a}
CQ	5.09 ± 0.11^{b}	0.8372	27.6 ± 3.10^{b}
MX	11.86 ± 0.88^{c}	0.6203	20.4 ± 2.12^{c}
AQ	10.75 ± 0.15^{c}	0.6562	22.1 ± 3.22^{c}
AQ-MX	4.59 ± 0.02^{b}	0.8531	30.8 ± 4.07^{b}
Note: PC: Parasitized Control; CQ: Chloroquine (Positive control); AQ: Amodiaquine; MX: Moxifloxacin, AQ-MX: Amodiaquine/Moxifloxacin; ^{a,b,c}Values with difference superscripts down the column significantly differ at $p<0.05$ (ANOVA)			

Table 1: Curative effect of amodiaquine-moxifloxacin on mice infected with *Plasmodium berghei.*

Suppressive effect of amodiaquine-moxifloxacin on mice infected with *Plasmodium berghei*.

AQ-MX decreased percentage parasitemia with significant difference observed at $p<0.05$ when compared to AQ or MX [17]. The inhibitions produced by AQ, MX and AQ-MX represent 72.40%, 70.63% and 94.38%, respectively, while CQ produced 93.80% inhibition. AQ-MX prolonged MST significantly ($p<0.05$) when compared to individual doses of AQ and MX (Table 2).

Treatment	% Parasitamia	% Inhibition	MST (Days)
PC	27.86 ± 2.10^{a}	0	9.23 ± 0.13^{a}
CQ	1.72 ± 0.20^{b}	0.938	30.26 ± 3.17^{b}
MX	8.18 ± 0.63^{c}	0.7063	25.14 ± 3.44^{c}
AQ	7.69 ± 0.16^{c}	0.724	28.73 ± 3.40^{c}
AQ-MX	1.58 ± 0.04^{b}	0.9438	33.08 ± 7.03^{b}

Note: PC: Parasitized Control, CQ: Chloroquine (Positive control), AQ: Amodiaquine, MX: Moxifloxacin, AQ-MX: Amodiaquine/Moxifloxacin; ^{a,b,c}Values with difference superscripts down the column significantly differ at $p<0.05$ (ANOVA)

Table 2: Suppressive effect of amodiaquine-moxifloxacin on mice infected with *Plasmodium berghei*.

Prophylactic effect of amodiaquine-moxifloxacin on mice infected with *Plasmodium berghei*.

Treatment with AQ-MX decreased percentage parasitemia with significantly ($p<0.05$) when compared to individual doses of AQ and MX [18]. AQ, MX and AQ-MX produced 72.27%, 75.22% and 97.76% inhibitions while CQ produced 96.25% inhibition. AQ/MX significantly ($p<0.05$) prolonged MST when compared to individual doses of AQ and MX (Table 3).

Treatment	% Parasitemia	% Inhibition	MST (Days)
PC	22.25 ± 0.68^{a}	0	9.61 ± 0.16^{a}
CQ	0.83 ± 0.20^{b}	0.9625	34.15 ± 3.01^{b}
MX	6.17 ± 0.77^{c}	0.7227	27.54 ± 3.12^{c}
AQ	5.51 ± 0.01^{c}	0.7522	29.86 ± 3.21^{c}
AQ-MX	0.50 ± 0.01^{d}	0.9776	37.71 ± 5.10^{b}

Note: PC: Parasitized Control; CQ: Chloroquine (Positive control); AQ: Amodiaquine; MX: Moxifloxacin; AQ-MX: Amodaiquine/Moxifloxacin; ^{a,b,c,d}Values with difference superscripts down the column significantly differ at $p<0.05$ (ANOVA)

Table 3: Prophylactic effect of amodiaquine-moxifloxacin on mice infected with *Plasmodium berghei*.

Effect of amodiaquine-moxifloxacin on hematological indices on mice infected with *Plasmodium berghei*.

RBCs, PCV and HB were significantly ($p<0.05$) increased whereas WBCs were significantly ($p<0.05$) decreased in *Plasmodium berghei*-infected mice when compared to normal control [19]. But treatment with AQ-MX significantly increased RBCs, PCV and HB and significantly decreased WBCs when compared to individual doses of AQ and MX at $p<0.05$ (Table 4 and Figure 1).

Treatment	RBCs ($\times 10^{6}$)	WBCs (cells/L)	PCV (%)	HB (g/dL)
NC	6.85 ± 0.02^{a}	4.76 ± 0.40^{a}	58.54 ± 5.18^{a}	15.64 ± 0.38^{a}
PC	2.00 ± 0.46^{b}	12.94 ± 0.11^{b}	20.56 ± 3.10^{b}	6.36 ± 0.26^{b}
CQ	5.67 ± 0.73^{c}	5.35 ± 0.30^{c}	49.61 ± 6.35^{c}	14.27 ± 0.41^{c}
MX	3.10 ± 0.27^{d}	9.63 ± 0.52^{d}	31.17 ± 3.85^{d}	10.01 ± 0.14^{d}
AQ	3.36 ± 0.17^{d}	8.77 ± 0.36^{d}	34.74 ± 4.98^{d}	10.50 ± 0.47^{d}
AQ-MX	5.94 ± 0.56^{c}	5.00 ± 0.30^{c}	52.03 ± 5.13^{c}	14.95 ± 1.33^{c}

Note: NC: Normal Control; PC: Parasitized control; CQ: Chloroquine (Positive control); AQ: Amodiaquine; MX: Moxifloxacin; AQ-MX: Amodiaquine/Moxifloxacin; RBCs: Red Blood Cells; WBC: White Blood Cells; PCV: Packed Cell Volume; HB: Hemoglobin; ^{a,b,c,d}Values with difference superscripts down the column significantly differ at $p<0.05$ (ANOVA)

Table 4: Effect of amodiaquine-moxifloxacin on hematological indices of mice infected with *Plasmodium berghei*.

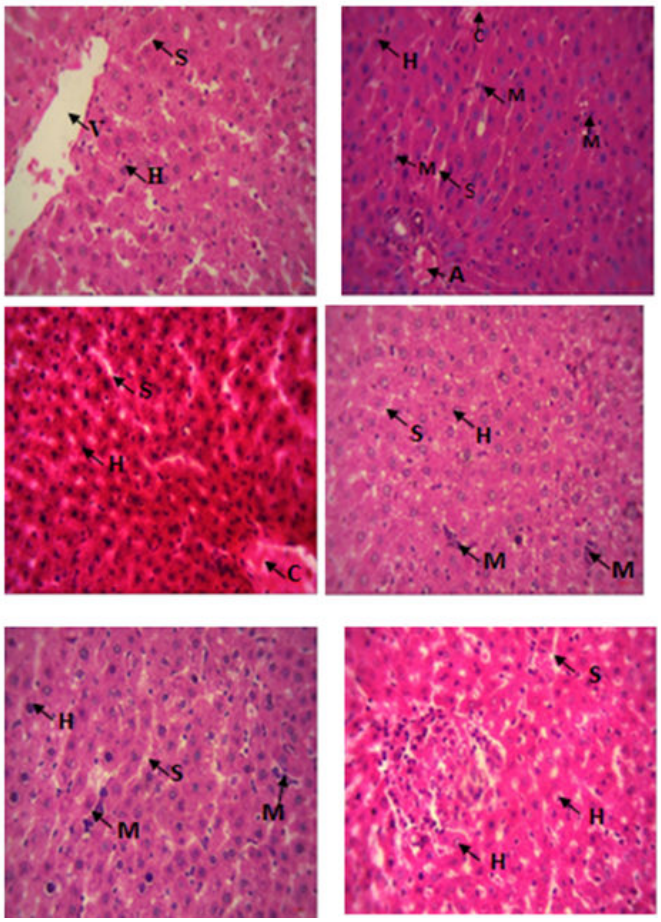

Figure 1: Control, parasitized control, standard control, amodaiquine (10 mg/kg)-treated mice, moxifloxacin (6 mg/kg)-treated mice, amodaiquine-moxifloxacin. H: Hepatocytes; C: Congested central vein; V: Central Vein; M: Merozoites; S: Sinusoids; A: Hepatic Artery.

Discussion

Malaria has caused notable health and economic challenges in the world especially in sub-Saharan Africa and South East Asia. Based on the WHO, in 2012, 207 million malaria cases and 627,000 malaria related deaths occurred in the world with cases (80%) and deaths (90%) in Sub-Saharan Africa [20]. In malarial therapy, combination therapy remains a good approach because it enhances the probability of sustained efficacy in the advent of parasite resistance to one agent. However, emerging Plasmodium resistance to some currently used antimalarial drugs warrants the search for new partners, which can be achieved through drug repurposing. Drug repositioning or the screening of existing drugs for new uses, affords an attractive, alternate and valid paradigm for drug discovery. This study explored whether MX can be repurposed as a partner drug with AQ for the treatment of malaria. The malaria parasites that cause malarial infection in humans are not able to invade non-primate animals. So, rodent malaria parasites are usually employed for the *in-vivo* assessment of antimalarial drug candidates. *P. berghei*, a rodent malaria parasite was used because it has been used for the screening of many conventional antimalarial drugs. This study selected the *in-vivo* malaria model because it allows pro-drug effect and the immune function in controlling malarial infection. Also its life cycle stages are clearly observed on smears due to non-adherence with endothelial cells. The four-day curative test which allows for established infections and the suppressive test used for this study are widely used methods for the screening potential antimalarial agents. In this study, curative, suppressive, prophylactic antiplasmodial assessments of AQ-MX showed reductions in percentage parasitemia levels. The prevention of malaria-related mortality is one of the most essential goals of malaria treatment, therefore an antimalarial drug candidates is expected to prevent of malaria-related mortality AQ-MX reduced mortality in treated mice through the prolongation of MST. Malaria infection is a common cause of anemia, which is associated with death especially among children. One of the essentials of malaria treatment is the prevention of malaria related anemia. AQ-MX conspicuously prevent anemia in treated mice, which was characterized by increased RBCs, HB and PCV levels.

Conclusion

One of the challenges in malaria treatment is the liver stage of malarial infection. It is imperative for an antimalarial candidate drug to be effective against liver stage of malarial infection. In the current study, AQ-MX eradicates liver *Plasmodium berghei* infection and restored liver histology. Interestingly, AQ-MX produced antiplasmodial effect similar to CQ the reference drug used for this study. The current study observed that the antiplasmodial effect of AQ-MX was additive, which may be attributed to the ability of MX to augment the antiplasmodial activity of AQ. The mechanism of action by which MX inhibits Plasmodium parasites in not clear. But studies suggested that it produces antiplasmodial activity by targeting the gyrase of parasites an enzyme essential for apicoplast DNA replication. MX seems effective as a partner drug to AQ, therefore AQ-MX may be used for the treatment of malaria.

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