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ORIGINAL ARTICLE

Antimalarial activity of amodiaquine-moxifloxacin in parasitized mice

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Abstract

Background: The search for new partner drugs to increase the therapeutic activities of the 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.

Methods: Adult Swiss albino mice (28-35g) of both sexes, randomly grouped and inoculated with Plasmodium berghei were used. Plasmodium berghei were used. The mice were treated orally with AQ (10 mg/kg/day), MX (6 mg/kg/day) and AQ-MX, respectively using the curative, prophylactic and suppressive protocols. Chloroquine (CQ) (10 mg/kg/day) was used as the positive control. 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 the treated mice.

Results: 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 65.62 %, 62.03% and 85.31% parasitemia inhibitions, respectively whereas CQ produced 83.72 % 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 significantly increased hemoglobin, red blood cells, and packed cell volume and significantly decreased white blood cells at $p<0.05$ when compared to AQ or MX. AQ-MX eradicates liver merozoites.

Conclusions: MX may be an effective partner drug with AQ for malaria treatment.

Keywords: Amodiaquine, Moxifloxacin, Antimalarial, Plasmodium, Mice.

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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 programme in 1950s and 1960s (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 (2) toward the prevention and eradication of malaria (3).

One of the challenges in the treatment of malaria especially in the tropics is the emergence of resistant to most antimalarial drugs (4,5). Combination therapies especially artemisinin combination therapies (ACTs), which combine artemisinins with partner drugs were introduced to overcome the incidence of *Plasmodium* resistance. ACTs have produced remarkable success against *Plasmodium* resistance however, there is gradual emergence of *Plasmodium* resistance to ACTs (4,5). The de novo emergence of resistance can be prevented by the continual exploration of new antimalarial drug combinations. New combinations can delay or slow the emergence and spread of resistance by eliminating resistant mutants except those that carry two different mutations (6-8).

Amodiaquine (AQ) is an orally active 4 aminoquinoline derivative with antimalarial and anti-inflammatory properties similar to chloroquine (9). It is used for the treatment of malaria including uncomplicated *Plasmodium falciparum* malaria (10). AQ has been used with good outcomes as a partner drug with artesunate, sulfadoxine and pyrimethamine to reduce the risk of resistance (10, 11). Moxifloxacin (MX) belongs to the fluroquinoline family, it is a broad spectrum antibiotic that is active against gram positive and gram negative bacteria (12, 13). In bacteria, it inhibits DNA gyrase and topoisomerase IV (14). MX has been associated with antiplasmodial activity against Plasmodium falciparum strains with the suggestion that it may serve as a viable partner drug with artemisinins and other antimalarial drugs (15).This study assessed whether MX could be a viable partner drug with AQ in *Plasmodium berghei* (*P. berghei*)-infected mice.

MATERIALS AND METHODS

Animals, drugs and parasites

Ninety Swiss albino mice of both sexes (28-35 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. 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.

This study was approved by Research Ethics Committee of the Department of Pharmacology/Toxicology, Faculty of Pharmacy, Niger Delta University, Bayelsa State (Approval date: 10.01.2022, Approval number: NDU/PHARM/PCO/AEC/082)

Dose selection

Chloroquine (CQ) (10mg/kg) (16), MX (6 mg/kg) (17) and AQ (10 mg/kg) (18) were used for the study.

Parasite inoculation

Donor mice infected with CQ-sensitive strain of *P. berghei* (NK65) used were obtained from Nigerian Institute of Medical Research, Yaba, Lagos. Stock inoculation containing 1×10^7 *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 1×10^7 *P. berghei* was inoculated into each mouse through intraperitoneal (ip) route.

Determination of antiplasmodial activity

Determination of curative antiplasmodial activity

The method described by Ryley and Peters (1970) (19) was used. Thirty adult Swiss albino mice randomized into 6 groups containing 5 mice per group were used. The mice were parasitized with 1×10^7 *P. berghei* (i.p) except for the normal control. After 3 days, the mice were treated per oral (p.o) with AQ (10mg/kg), MX (6mg/kg) and AQ-MX daily for 4 days. The parasitized and normal controls were 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. The number of parasitized red blood cells (RBCs) were counted against the total number of RBCs in a field. Percentage parasitemia and inhibitions were calculated as shown below.

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

$$\% \text{ Inhibition} = \frac{(\% \text{ Parasitemia of negative control} - \% \text{ Parasitemia of treated group}) \times 100}{\% \text{ Parasitemia of negative control}}$$

Determination of suppressive antiplasmodial activity

The method described by Knight and Peters (1980) (20) was used. Thirty adult Swiss albino mice were randomly divided into 6 groups of 5 mice per group and parasitized with 1×10^7 *P. berghei* ip for 3 h. Thereafter, the mice were treated per oral (p.o) with AQ (10mg/kg), MX (6mg/kg) and AQ-MX daily for 4 days. The parasitized and normal controls were treated with normal saline (0.2 mL), while the positive (Standard) control was treated with CQ (10 mg/kg) daily for 4 days. On day 5, blood samples were collected from the tail and thin films were prepared on slides. Percentage parasitemia and inhibitions were calculated as shown above.

Determination of prophylactic antiplasmodial activity

The method described by Peters (1975) (21) was used for prophylactic test. Thirty adult Swiss albino mice randomized into 6 groups containing 5 mice per group were used. The mice were treated per oral (p.o) with AQ (10mg/kg), MX (6mg/kg) and AQ-MX daily for 4 days. The parasitized and normal controls were treated with normal saline (0.2 mL) while the positive (Standard) control was treated with CQ (10 mg/kg) daily for 4 days. On day 5, except for the normal control the mice were inoculated with 1×10^7 *P. berghei* ip and treatment continued for 4 days. Thereafter, blood samples were collected from the tail and percentage parasitemia and inhibitions were determined as stated above

Determination of mean survival time

The mice in the control and the treated groups were observed for mortality and expressed in days. Mortality expressed as mean survival time (MST) was calculated as shown below.

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

2.4.5 Evaluation of hematological 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) and white blood cells (WBCs) using an auto analyzer.

Statistical analysis

Data expressed as 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 $P< 0.05$.

RESULTS

Curative antiplasmodial 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 parasitemia inhibitions of 65.62%, 62.03%, and 85.31%, respectively, while CQ produced 83.72% parasitemia inhibition **(Table1).** Treatment with AQ-MX significantly prolonged MST when compared to AQ or MX with significance observed at $p<0.05$ **(Table 1).**

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

Treatment	% Parasitemia	% Inhibition	MST(Days)
PC	31.26±1.23a	0.0	9.05±0.97 a
CQ	5.09±0.11b	83.72	27.6±3.10 b
AQ	10.75±0.15c	65.62	21.1±3.22 c
MX	11.86±0.88c	62.03	20.4±2.12 c
AQ-MX	4.59±0.02b	85.31	30.8±4.07 b

PC: Parasitized control, CQ: Chloroquine (Positive control), AQ: Amodiaquine, MX: Moxifloxacin, AQ-MX: Amodiaquine-Moxifloxacin. Values with difference superscripts down the column significantly differ at p<0.05 (ANOVA: Analysis of variance)

Suppressive antiplasmodial 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. The parasitemia inhibitions produced by AQ, MX and AQ-MX represent 72.40%, 70.63%, and 94.38%, respectively, while CQ produced 93.80% parasitemia inhibition **(Table 2).** AQ-MX prolonged MST significantly (p<0.05) when compared to individual doses of AQ and MX **(Table 2)**

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

Treatment	% Parasitemia	% Inhibition	MST(Days)
PC	27.86±2.10 a	0.00	9.23±0.13a
CQ	1.72±0.20 b	93.80	30.26±3.17b
AQ	7.69±0.16 c	72.40	28.73±3.40c
MX	8.18±0.63 c	70.63	25.13±3.66c
AQ-MX	1.58±0.04 b	94.38	33.08±7.03b

PC: Parasitized control, CQ: Chloroquine (Positive control), AQ: Amodiaquine, MX; Moxifloxacin, AQ-MX: Amodiaquine-Moxifloxacin. MST: Mean survival time, Values with difference superscripts down the column significantly differ at p<0.05 (ANOVA: Analysis of variance)

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

Treatment with AQ-MX decreased percentage parasitemia with significance at p<0.05 when compared to individual doses of AQ and MX **(Table 3).** AQ, MX, and AQ-MX produced 75.22%, 75.27% and 97.76% parasitemia inhibitions while CQ produced 96.25% parasitemia inhibition **(Table 3).** AQ-MX significantly (p<0.05) prolonged MST when compared to individual doses of AQ and MX **(Table 3).**

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

Treatment	% Parasitemia	% Inhibition	MST(Days)
PC	22.25±0.68 a	0.0	9.61±0.16 a
CQ	0.83±0.20 b	96.25	34.15±3.01b
AQ	5.51±0.01 c	75.22	29.86±3.00c
MX	6.17±0.77 c	72.27	27.54±3.21c
AQ-MX	0.50±0.01 d	97.76	38.71±5.10b

PC: Parasitized control, CQ: Chloroquine (Positive control), AQ: Amodiaquine, MX; Moxifloxacin, AQ-MX: Amodaiquine-Moxifloxacin. MST: Mean survival time, Values with difference superscripts down the column significantly differ at p<0.05 (ANOVA: Analysis of variance)

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 *P. berghei*-infected mice when compared to the normal control **(Table 4).** However, 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).**

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

Treatment	RBCs (x106)	WBCs (cells/L)	PCV (%)	HB (g/dL)
NC	6.85±0.02 a	4.76±0.40 a	58.54±5.18a	15.64±0.38 a
PC	2.00±0.46 b	12.94±0.11b	20.56±3.10 b	6.36±0.26 b
CQ	5.67±0.73 c	5.35±0.30 c	49.61±6.35c	14.27±0.41c
AQ	3.36±0.17 d	8.77±0.36 d	34.74±4.98d	10.50±0.47d
MX	3.10±0.21 d	9.63±0.52 d	31.17±3.85d	10.01±0.12d
AQ-MX	5.94±0.56 c	5.00±0.30 c	52.03±5.13 c	14.95±1.33c

NC: Normal control, PC: Parasitized control, CQ: Chloroquine (Positive control), AQ: Amodiaquine, MX: Moxifloxacin, AQ-MX: Amodiaquine-Moxifloxacin RBCs: Red blood cells, WBCs: White blood cells, PCV: Packed cell volume, HB: Hemoglobin, Values with difference superscripts down the column significantly differ at p<0.05 (ANOVA: Analysis of variance)

DISCUSSION

Malaria has caused notable health and economic challenges in the world especially in sub-Saharan Africa and South East Asia (22). Based on WHO report, in 2012, 207 million malaria cases and 627,000 malaria related deaths occurred in the world (23). In the treatment of malaria, combination therapy remains a good approach, because it enhances the possibility of sustained efficacy in the advent of parasite resistance to one agent (24). However, emerging *Plasmodium* resistance to some currently used antimalarial drugs warrants the search for new partner drugs, 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 (25). This study explored whether MX can be repurposed as a partner drug with AQ for the treatment of malaria. The malaria parasites that cause infection in humans are not able to invade non-primate

animals, therefore rodent malaria parasites are usually employed for the *in-vivo* assessment of antimalarial drug candidates (26). *P. berghei*, a rodent malaria parasite was used, because it has been used for the screening of many conventional antimalarial drugs (19). This study selected the *in- vivo* malaria model, because it allows pro-drug effect and the immune function in controlling malaria infection (27). Also, it allows for parasites life cycle stages to be clearly observed on smears due to non-adherence with endothelial cells (28). A 4-day curative test for established infections and a 4- day suppressive test for early infections were used (29). 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 (30, 31), therefore an antimalarial drug candidate is expected to prevent malaria–related mortality (32). In this study, AQ-MX reduced mortality in the treated mice as shown by prolonged MST. Malaria infection is a common cause of anemia, which has been associated with death especially among children. One of the essentials of malaria treatment is the prevention of malaria-related anemia (33, 34). AQ-MX conspicuously prevent anemia in the treated mice, which was characterized by increased RBCs, HB and PCV levels. One of the challenges in malaria treatment is the liver stage of malaria infection. It is imperative for an antimalarial candidate drug to be effective against liver stage of malaria infection (35). This study, observed merozoites, and central vein congestion in *P. berghei* -infected mice. Udongkang *et al.* reported similar findings in *P. berghei*-infected mice (36). However, treatment with AQ-MX decreased liver merozoites and restored liver histology. Interestingly, AQ-MX produced antiplasmodial effect similar to CQ the standard drug used for this study. The current study observed that the antiplasmodial effect of AQ-MX was additive. The mechanism of action by which MX inhibits *Plasmodium* parasites in not clear. However, studies suggested that it produces antiplasmodial activity by targeting the gyrase of parasites, which is an enzyme essential for apicoplast DNA replication (37, 38).

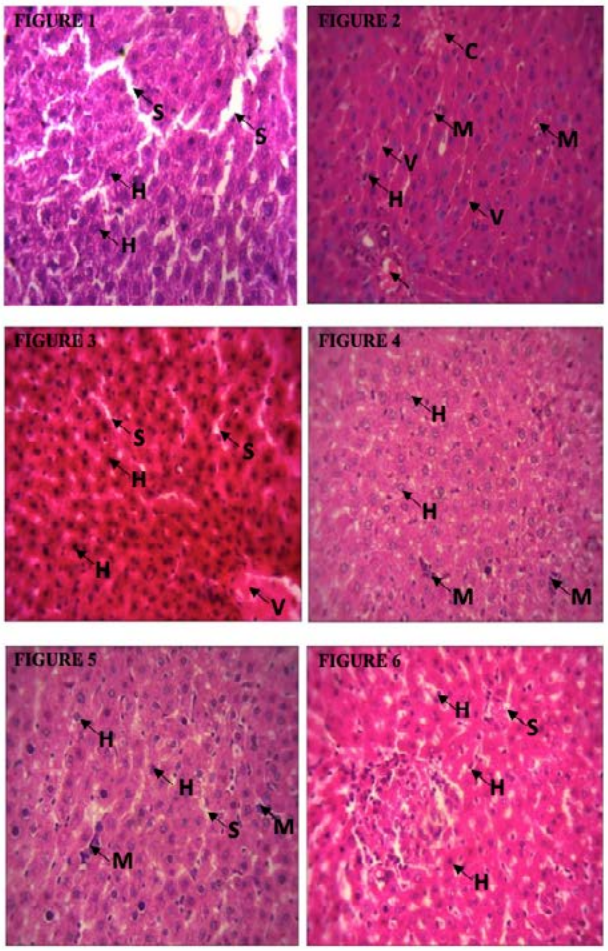

Figure 1 (Control), Figure 2 (Parasitized control), Figure 3 (Standard control), Figure 4 (Amodaiquine [10 mg/kg]-treated mice), Figure 5 (Moxifloxacin [6mg/kg] -treated mice), Figure 6 (Amodiaquine-Moxifloxacin). H: Hepatocytes, C: Congested central vein, V: Sinusoidal congestion, M: Merozoites, S: Sinusoids, A: Hepatic artery.

MX seems effective as a partner drug with AQ, therefore AQ-MX may be used for the treatment of malaria.

Declarations

The authors received no financial support for the research and/or authorship of this article. There is no conflict of interest.

This study was approved by Research Ethics Committee of the Department of Pharmacology/Toxicology, Faculty of Pharmacy, Niger Delta University, Bayelsa State (Approval date: 10.01.2022, Approval number: NDU/PHARM/PCO/AEC/082)

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