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Research Article

Antimalarial-Antibiotic Combination for Malaria: Evaluation of the Potential Toxicity and Safety of the Combinations of Amodiaquine-Ciprofloxacin and Amodiaquine-Rifampicin

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Abstract

The combinations of antibiotics with standard anti-malarial drugs have been documented as potentially valuable chemotherapeutic options for the management of drug-resistant malaria infections. However, the potential adverse effects and/ or toxic manifestation of the combinations remain to be addressed. This study was designed to investigate the probable toxicological effect of the combination of amodiaquine with rifampicin or ciprofloxacin. Forty-two Swiss mice with average weight of 24 g were divided into six groups. They were treated orally with amodiaquine (10 mg/kg), ciprofloxacin (160 mg/kg), rifampicin (15 mg/kg), amodiaquine plus rifampicin or ciprofloxacin and 0.2 mL normal saline solution respectively. Biochemical and hematological analysis of blood samples were performed using standard procedures, while physical toxicological manifestation was monitored daily. Amodiaquine alone, and amodiaquine/ciprofloxacin significantly increased the activity of Alkaline phosphatase (ALP) [$P<0.05$]. Ciprofloxacin alone and in combination with amodiaquine significantly ($P<0.05$) decreased the level of aspartate aminotransferase (AST) compared to the control (58.28 U/L vs 373.7 U/L respectively). The activity of alanine aminotransferase (ALT) was significantly decreased in animals that received rifampicin alone ($P<0.05$). Uric acid concentration and g-glutamyl transferase (GGT) were significantly increased by the combination of amodiaquine and rifampicin. Mice in ciprofloxacin/ amodiaquine treatment group manifested adverse effects such as weight loss, sluggishness and loss of fur. Study animals survived beyond the duration of study despite noticeable known adverse effects associated with the individual drugs. These effects may not constitute a deterrent in the consideration of the potential usefulness of the antibiotic and antimalarial combinations.

Key Words: Malaria, Toxicity, Combination therapy, Amodiaquine, Ciprofloxacin, Rifampicin

INTRODUCTION

Malaria is a debilitating disease which affects millions of lives causing great socio-economic deprivation and huge mortality in endemic regions. About half of the world's population (3.3 billions) is at risk of acquiring malaria, with an estimated annual average of over 200 million cases reported (WHO, 2021). Global annual malaria mortality in 2020 was approximately 627,000, the vast majority being children under five years old and pregnant women living in sub-Saharan Africa (WHO, 2021). Different interventions in combating malaria have culminated in the dramatic reduction of the malaria burden within the last two decades; with approximately 10.6 million would be malaria-related deaths averted (WHO, 2021).

This progress in reducing the malaria burden is now being challenged by a number of factors, topmost of which is emergence and re-emergence of parasite drug resistance (WHO, 2020, Balikagala *et al*., 2021). Increasing cases of parasite resistance to artemisinin-based combination therapy (ACT) with high rates of treatment failures have been reported in Southeast Asia (Gbotosho and Akinola, 2015; Paloque *et al*., 2016; Imwong *et al*., 2017; Nsanzabana, 2019; Mathenge *et al*., 2020) and now in Africa (Balikagala *et al*., 2021; White, 2021).

The implications of artemisinin resistance are far-reaching and may cause a potential clinical catastrophe (White, 2021), especially in Africa, where more than 90% of all malaria cases are recorded (Alonso and Noor, 2017, WHO, 2021). Furthermore, the possibility of a resurgence in regions where malaria has been eradicated due to factors such as climate change, population movements, insufficient funding, expired strategies, lapses in surveillance, among others, cannot be overlooked (Lover and Coker, 2013; Shretta *et al*., 2017;

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WHO, 2019). Thus, there is need for the development of alternative treatment strategies and/ or development of newer therapies that can combat and counter the spread of drug-resistant malaria parasites.

Although, there is continuing progress in the development of more effective antimalarial drugs, the lag time between drug developments, registration, change of national treatment policy and substantial large-scale production may infer an inescapable and unavoidable delay before affordable substitutes to ACTs are widely available (Lubell *et al*., 2014). A potential approach is to reposition and find new uses for drugs that have been in use for other indications (Nosengo, 2016; Rusha *et al*., 2020). This approach may also offer exploration of new combinations regimens that may target different bio-processes within the parasites (Badejo *et al*., 2014).

In a bid to repurpose the use of antibiotics as potential regimen in the chemotherapy of malaria, studies have uncovered several antibiotics that possess antimalarial activity both in vitro and in vivo (Andrade *et al*., 2007; Gbotosho *et al*., 2012; Badejo *et al*., 2014; Sahu *et al*., 2014). Focus had been on tetracyclines, fluoroquinolones, macrolides and their derivatives, however, many other widely used antibiotics belonging to older families, such as co-trimoxazole, fusidic acid and quinolones have been investigated for their potential use in malaria (Gaillard *et al*., 2016). Newer antibiotics such as tigecycline, mirincamycin and ketolide agents have also been investigated for their potential future use for malaria chemotherapy (Gaillard *et al*., 2016).

Quinolone analogues have been documented as potent alternative drugs for malaria (Pradines *et al*., 2001; Mahmoudi *et al*., 2003; Dahl and Rosenthal 2007; Beteck *et al*., 2014). They target several stages of the malaria parasite life cycle via different modes of action from most currently used drugs (Gaillard *et al*., 2016). The most commonly used quinolones are the fluoroquinolone antibiotics; Norfloxacin, ofloxacin, and ciprofloxacin (CFX). Many studies have documented the potential usefulness of ciprofloxacin as an anti-malarial in vivo in experimental animal models where it was reported to possess a potentiating action when used in combination with standard antimalarial drugs (Gbotosho *et al*., 2012; Falajiki *et al*., 2015, Ubulom *et al*., 2015). The mono-administration of ciprofloxacin showed a delayed killing effect on the parasites; so, they were considered to possess better antiparasitic effects when used in combination with rapidly acting antimalarial drugs.

Likewise, rifampin has been reported to be active against the murine malaria, *Plasmodium chabaudi* and *Plasmodium berghei* in vivo (Pukrittayakamee *et al*., 1994). Moreover, combination of rifampicin with isoniazid and co-trimoxazole was observed to be effective in patients with P. falciparum infections (Georg *et al*., 1999). Rifampicin may have a potential role in the management of malaria (Strath *et al*., 1993; Badejo *et al*., 2014)

The use of antibiotics with antimalarial properties may represent a potentially valuable chemotherapeutic option in the fight against the spread of artemisinin-resistant parasites, particularly in combination with standard antimalarial drugs (Sahu *et al*., 2014). The potential for this combination to be developed as an antimalarial regimen cannot be overlooked. However, detailed pharmacokinetics and toxicological studies are required to validate the beneficial interaction of the antimalarial/antibiotic combination (Falajiki *et al*., 2015). This study was therefore designed to evaluate the possible toxicological effects of these antimalarial/antibiotic combinations in order to further underscore their potential usefulness in the fight against malaria.

MATERIALS AND METHODS

Animals: Forty-two female Swiss mice with an average weight of 24 g were used for the experiment. 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.

Drug preparation: Ciprofloxacin (CFX) and Rifampicin (RIF) were generously provided by Bond Chemicals Ltd., Awe, Oyo State, Nigeria. Amodiaquine (AQ) was purchased from Sigma, St. Louis, MO, USA. Stock solutions of amodiaquine and ciprofloxacin were dissolved in distilled water and working solutions of desired concentrations were prepared by dilution. Rifampicin was dissolved in 10% dimethylsulphoxide (DMSO); required quantity was weighed, dissolved in 1 ml DMSO then warmed for 10 minutes in a water bath at 600 ^{o}C. The resultant solution was made up to 10 ml with water. All drugs were prepared 24 hours prior to use.

Treatment Groups: Forty-two (42) Swiss mice were used for each experiment. Study animals were divided into six groups with six mice per group in groups I-V, while group VI had twelve (12) animals and served as the study controls. On day one of study, animals in groups I-V were treated with the following drug regimen; 10 mg/kg AQ (Group 1), 160 mg/kg CFX (Group 2), 15 mg/kg RIF (Group 3), 10 mg/kg AQ + 160 mg/kg CFX (Group 4), or 10 mg/kg AQ + 15 mg/kg RIF (Group 5). Animals in group VI received 0.2 mL normal saline. Amodiaquine was administered for three days, ciprofloxacin for five days and rifampicin for seven days. The animals were observed for manifestations of side/adverse effects for a period of 35 days.

Twenty-four (24) hours after the administration of the last dose of amodiaquine, 2 animals from the AQ treatment group and 2 from the control group were euthanized. Blood was obtained through cardiac puncture into plain bottles for biochemical assays of liver and kidney parameters, while heparinized vacutainer tubes were utilized for blood samples intended for hematological analysis.

The sample collection procedure described above was repeated on day 6 for animals in Groups II (CFX), IV (AQ + CFX) and VI (control), and also on day 8 for Groups III (RIF), V (AQ+RIF) and VI (control). All samples were analyzed relative to the controls.

Hematological parameters: The white blood cell (WBC), red blood cell (RBC), hemoglobin (HGB), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet (PLT) were determined using a fully automated hematology analyzer (Pentra-XL 80, Horiba ABX, USA).

Biochemical analysis: The procedure described by Yakubu *et al* (2009), was adopted in the preparation of serum used for the biochemical analysis. The blood samples were allowed to clot and then centrifuged at 3,000 g for 10 minutes and serum was aspirated using a Pasteur pipette into sample bottles. Liver and kidney biochemical analysis were carried on the processed samples to assay the changes in the levels of the following parameters; creatinine, calcium, phosphorus, albumin, bilirubin, alkaline phosphatase (ALP), g-glutamyl transferase (GGT), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) by spectrophotometric determination using assay kits obtained from Roche Diagnostics GmbH (Mannheim, Germany).

Statistics: Data were expressed as the mean ± SEM. Data were statistically analyzed using a one-way analysis of variance (ANOVA). Means were separated by Duncan's Multiple Range Test. Statistical significance was determined at a level of $P < 0.05$.

RESULTS

Physical toxicological manifestations

The animals were observed daily for signs of toxicity and weighed on alternate days. Physical changes observed were the toxic effects of the individual drugs and they included loss of appetite, weakness, sluggishness, loss of fur and weight loss.

Biochemical Analysis

Effects of amodiaquine (10 mg/kg) alone, ciprofloxacin (160 mg/kg) alone and the combination of amodiaquine plus ciprofloxacin on selected serum biochemical indices

Amodiaquine alone and the combination of amodiaquine and ciprofloxacin significantly increased the activity of ALP in mice when compared to the control group ($P<0.05$). In contrast, animals that received amodiaquine (10 mg/kg) alone had comparable levels of albumin, total protein and bilirubin relative to the control groups. Similarly, the concentration of creatinine and uric acid were not significantly altered (Table 1) by amodiaquine. Ciprofloxacin (160 mg/kg) administered alone significantly ($P<0.05$) reduced the activity of AST relative to the control. Similar outcomes *were* also observed with the combination of amodiaquine (10 mg/kg) plus ciprofloxacin (160 mg/kg) administered twice daily, which significantly ($P<0.05$) decreased the activity of AST to a mean value of 58.28 U/L, compared to the value in control group of animals (305.13 U/L) as seen in Table 2.

Effects of rifampicin alone and the combination of amodiaquine plus rifampicin on selected serum biochemical indices

The uric acid concentration was significantly ($P<0.05$) increased by rifampicin (15 mg/kg) alone and in combination with amodiaquine (10 mg/kg) (Table 3). The concentration of ALT was significantly ($P<0.05$) decreased in animals treated with 15 mg/kg rifampicin relative to the untreated control group.

The activity of ALP and GGT were significantly increased ($P<0.05$) in the amodiaquine plus rifampicin treated group of animals when compared to the control group of animals that received normal saline. All other parameters in the rifampicin treatment groups were not significantly affected when compared to the control as shown in table 3.

Table 1:
Effect of standard dose of amodiaquine (10 mg/kg) on biochemical parameters in experimental mice

	Control	AQ
Albumin (mg/dL)	40.97±0.19	42.47±0.50
Total protein (mg/dL)	23.44±0.49	23.89±0.25
Total Bilirubin (mg/dL)	9.70±0.04	11.18±0.04
Creatinine (mg/dL)	86.43±0.88	88.12±0.50
Uric acid (mg/dL) × 10	4.81±0.01	4.43±0.00
AST (U/L)	303.80±25.27	325.50±36.44
ALT (U/L)	68.66±2.32	64.29±0.93
ALP (U/L)	167.30±0.03	211.94±0.40**
GGT (U/L)	2.82±0.03	1.79±0.17

AQ (10 mg/kg) for 3 days, Mean ±SEM. **statistically significant ($P<0.05$), AST = aspartate aminotransferase, ALT= alanine aminotransferase, ALP = alkaline phosphatase and GGT = g-glutamyl transferase.

Table 2:
Effect of ciprofloxacin (160 mg/kg) alone or the combination of amodiaquine (10 mg/kg) and ciprofloxacin (160 mg/kg) on kidney and liver function

	Control	CFX	AQ +CFX
Albumin (mg/dL)	40.83±0.22	43.56±0.16	43.16±0.12
Total protein (mg/dL)	23.72±0.03	38.06±1.07	35.24±0.11
Total Bilirubin (mg/dL)	11.85±0.07	20.89±0.05	20.99±0.26
Creatinine (mg/dL)	87.56±3.66	90.39±0.09	94.79±0.03
Uric acid (mg/dL)× 10	4.73±0.01	5.71±0.01	4.46±0.00
AST (U/L)	305.13±22.08	70.35±5.748**	58.28±1.945**
ALT (U/L)	64.55±1.39	63.75±0.10	52.68±0.10
ALP (U/L)	373.74±0.69	343.33±0.046	58.51±0.47**
GGT (U/L)	3.55±0.03	6.33±0.03	7.53±0.00

AQ (10 mg/kg) for 3 days, CFX (160 mg/kg) for 5 days, RIF (30 mg/kg) for 7 days, Mean ±SEM. **statistically significant ($P<0.05$). AST = aspartate aminotransferase, ALT= alanine aminotransferase, ALP = alkaline phosphatase and GGT = g-glutamyl transferase.

Table 3:
Effect of rifampicin (15 mg/kg) alone or combination of amodiaquine (10 mg/kg) and rifampicin (15 mg/kg) on

kidney and liver functions

	Control	RIF	AQ+RIF
Albumin (mg/dL)	3.95±0.39	3.73±0.31	4.12±0.32
Total protein (mg/dL)	10.45±0.36	9.15±0.11	9.88±0.06
Total Bilirubin (mg/dL)	0.72±0.02	0.62±0.02	1.22±0.05
Creatinine (mg/dL)	47.93±2.19	49.06±1.86	47.78±0.16
Uric acid (mg/dL)× 10	0.15±0.05	0.76±0.06**	0.72±0.06**
AST (U/L)	88.54±2.89	112.80±0.24	110.53±4.51
ALT (U/L)	462.90±6.72	197.24±3.35**	506.90±4.67
ALP (U/L)	180.70±4.44	170.94±16.10	651.34±5.76**
GGT (U/L)	24.89±0.67	14.47±1.19	61.52±1.51**

AQ (10 mg/kg) for 3 days, CFX (160 mg/kg) for 5 days, RIF (30 mg/kg) for 7 days, Mean ±SEM. ** statistically significant (P <0.05). AST = aspartate aminotransferase, ALT= alanine aminotransferase, ALP = alkaline phosphatase and GGT = g-glutamyl transferase.

Table 4: Effects of combination of amodiaquine (10 mg/kg) with ciprofloxacin (160 mg/kg) and/or rifampicin (15 mg/kg) on hematological parameters

	Control	CFX +AQ	Rifampicin +AQ
WBC	3.20±0.30	1.00±0.9	2.30±0.60
RBC	7.37±0.46	7.20±1.20	5.94±1.01
HGB	10.20±0.09	8.40±0.60	7.75±1.35
HCT	37.85±4.15	41.70±2.89	27.35±5.55
MCV	51.20±2.40	46.80±1.92	45.75±1.55
MCH	13.90±1.00	15.80±0.80	13.05±0.05
MCHC	27.30±3.30	28.20±0.24	28.55±0.85
PLT	889.00±164.00	954.30±178.80	752.50±87.50
% LYM	92.60±0.00	85.50±14.40	81.60±1.90
LYM	3.50±0.00	2.00±0.06	1.85±0.45
RWD-SD	33.25±1.85	36.70±1.16	28.75±0.15
RDW-CV	18.55±0.55	25.70±1.71	15.55±0.75
PDW PL	7.10±0.40	8.70±0.67	6.25±0.65
MPV PL	6.00±0.30	6.90±0.17	5.45±0.35
P-LCR R	4.40±0.69	3.20±0.87	1.85±0.55

AQ (10 mg/kg) for 3 days, CFX (160 mg/kg) for 5 days, RIF (30 mg/kg) for 7 days, Mean ±SEM. RBC - Red blood cell count, WBC - White blood cell count, HGB - Hemoglobin levels, HCT - Total hematocrit, MCV - Mean corpuscular volume, MCH - Mean corpuscular hemoglobin, MCHC - Mean corpuscular hemoglobin concentration, PLT - Platelet count, LYM - Lymphocyte count, RDW - Red cell distribution width, PDW - Platelet distribution width, MPV - Mean platelet volume, PLCR - Platelet large cell ratio.

Hematology Analysis

There were no significant changes in hematological parameters in any of the treatment groups as shown in Table 4. The levels of RBC, PCV, MCV, MCH, MCHC and other hematological parameters examined were not significantly altered in all treatment groups.

Relative Organ Weight (ROW)

There were no significant changes in the relative organ weights of the animals in all the groups tested. The combinations reflected no significant alteration in the organs of the animals in comparison to their body weight (Table 5).

Table 5:
Effect of the drugs on Relative Organ Weight (ROW)

Organ	Kidney (x 10^{-3}) (g)	Liver (x 10^{-3}) (g)
Day 4		
Control	0.05±0.00	0.02 ± 0.00
AQ	0.07±0.00	0.01 ± 0.00
Day 6		
Control	0.06 ±0.00	0.02 ± 0.00
CFX	0.06±0.00	0.02 ±0.00
CFX+AQ	0.08 ± 0.01	0.02 ± 0.00
Day 8		
Control	0.06±0.01	0.02 ± 0.00
RIF	0.07±0.00	0.05 ± 0.00
RIF+AQ	0.09 ± 0.00	0.02 ± 0.00

AQ (10 mg/kg) for 3 days, CFX (160 mg/kg) for 5 days, RIF (30 mg/kg) for 7 days. Mean ±SEM.

DISCUSSION

The combination of antimalarial drugs plus antibiotics have been evaluated and documented in previous studies as potential therapeutics in the treatment of drug-resistant malarial infections (Falajiki *et al*., 2015; Gaillard *et al*., 2015; Badejo *et al.*, 2014). These studies however expressed toxicity concerns over selected doses of the antimalarial/antibiotic combinations. In the present study, kidney and liver function indices were evaluated in order to determine possible organ disruption and extent by the combination of antimalarial/antibiotic drugs proposed as potential regimen in combating malaria.

Amodiaquine administered alone to the study animals in standard dose regimen, resulted in higher levels of ALP, an indication of its hepatotoxic potential which has been previously documented in clinically review of liver injuries (EASL, 2019). This indication is however, not a stand-alone indicator of liver impairment as the levels of liver enzymes often return to normal states after cessation of drug use

(Devarbhavi, 2012). This assumption is supported by the survival of animals in the group beyond the duration of the study. The altered level of ALP observed with the combination of amodiaquine plus rifampicin may not be unconnected with the pharmacodynamic properties of rifampicin as an auto-inducer of metabolic enzymes (Tahara *et al*., 2019). These effects are evident from the increased concentration of ALP, which may occur as a result of infiltrations of hepatocytes into systemic circulation (Wintola *et al*., 2011). Interestingly, the levels of these enzymes were not altered by mono-administration of CFX or RIF suggesting that changes observed may be due to the effect of combination with AQ. This assertion correlates with previous report of a study where the mono-administration of azithromycin or rifampicin had no effect on the level of ALP (Hassanpour and Kheirkhah, 2015). The decline in ALP and AST levels observed in AQ + CFX groups of animals remains unexplained as both drugs are known to possess mild hepatotoxic potentials that increase levels of liver enzymes (Baloch *et al*., 2017). Nonetheless, ciprofloxacin is still an effective and safe antibiotic with very few isolated cases of hepatoxicity (Andrade and Tulkens, 2011)

The increase in the concentration of uric acid is often considered an early indicator of renal toxic effect of a substance (Ashafa *et al*., 2009). Therefore, the increase in the concentration of uric acid by rifampicin alone and its combination with amodiaquine can be associated with potential oxidative pressure induced by rifampicin on renal corpuscles (Heidari *et al*., 2014), which may affect the normal functioning of the nephrons at the tubular and glomerular levels (Ratliff *et al*., 2016). This correlates with a study where the co-administration of isoniazid/rifampicin elevated the levels of creatinine, urea and uric acid (Hussein *et al*., 2016) and was attributed to loss of bulk of kidney functions.

There was no significant alteration in the liver- and kidney-body weight ratios; this may indicate that the drugs and drug combinations did not affect the organs adversely as increase in organ-body weight ratio may be due to inflamed organs while a decrease in the ratio typifies cellular constriction within the organs (Adebiyi *et al*., 2016). Furthermore, there were no significant changes in hematological parameters in any of the treatment groups, even though amodiaquine has previously been reported to mediate agranulocytosis in sprague-dawley rats (Saka *et al*., 2012). Significant physiological changes, such as weight loss, sluggishness, loss of fur, behavioral changes and reduced physical activities were observed in all treated groups relative to the controls. These observed manifestations were similar to adverse events reported in previous studies on these combinations in the treatment of malaria-infected mice (Falajiki *et al*., 2015; Badejo *et al*., 2014). Interestingly, the recovery to normal physiological states was observable 24 hours post drug administration and all study animals survived beyond the 35 days duration of study. This is in contrast to studies with similar dosage regimen, where the study animals were infected with malaria parasites (Falajiki *et al*., 2015; Badejo *et al*., 2014). Thus, the reported number of deaths in the previous studies may be solely attributable to the malaria infection in study animals, with little influence of the side/adverse effects of the drug combinations.

In conclusion, despite the tolerability of the antibiotics and antimalarial drugs used in this study, the findings reveal that the combinations are capable of adversely affecting the hepatic and renal functions of mice. However, most of the toxic effects observed are not uncommon with the clinical use of the individual drugs. Moreover, the animals recovered from the physical manifestation of the toxic effects of the drugs, like loss of appetite, weakness, sluggishness, loss of fur and weight loss, observed during the study period.

Amodiaquine, rifampicin and ciprofloxacin are not completely free of adverse effects; these drugs however continue to be in use for their different indications as they are well tolerated. This implies that the biochemical changes observed in this study should not deter the pursuit of further studies on antibiotic/antimalarial drug combinations. However, additional studies are required to ascertain the usefulness of this combination in the treatment of malaria in the presence of liver and renal impairment.

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