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Research Article

The Impact of Rifampicin on Antimalarial Activity of Piperaquine and Piperaquine/Dihydroartemisinin Combination.

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Resistance to the available artemisinin-based combination therapy continues to threaten the gains made through global efforts to control and eliminate malaria, one of the most severe public health problems in sub-Saharan Africa. In this study, the therapeutic efficacy of combinations of rifampicin with piperaquine or, piperaquine/dihydroartemisinin was evaluated in a murine model of malaria. A modification of Peter's four-day suppressive test was used to evaluate chemosuppression of parasitemia, parasite clearance time (PCT), parasite recrudescence time (PRT), survival time and survival rate by the selected drug combinations. Fifty Swiss albino mice weighing 18 – 22g were intravenously inoculated with 1 x 107 chloroquine-resistant *P. berghei* (ANKA) and assigned to ten treatment groups (n=5); rifampicin 15mg/kg or 30mg/kg, piperaquine (16mg/kg), piperaquine/rifampicin 15mg/kg or 30mg/kg, piperaquine (16mg/kg)/dihydroartemisinin (2mg/kg), piperaquine/dihydroartemisinin/rifampicin 15mg/kg or 30mg/kg. A group was assigned to receive standard dose of chloroquine (10mg/kg), while the control distilled water. All drugs were administered orally over a 3-day period, and animals were followed up for 42 days. Parasitemia was suppressed by 100% between day 4 to 11 post-infection in all treatment groups, except in the two controls, and rifampicin-alone groups. The PCT and survival rates of animals that received rifampicin-based combinations were not significantly different ($p>0.05$) compared with piperaquine/dihydroartemisinin treated animals. Animals that received rifampicin-based combinations had longer PRT ranging from 29.6 – 33.0 days, compared with 26.2 days in piperaquine/dihydroartemisinin treated animals. The antimalarial efficacy of rifampicin-based combination appeared superior to piperaquine/dihydroartemisinin, hence a promising antimalarial combination with the potential of mitigating resistance to the component drugs.

Key Words: Chemosuppression of parasitemia, Malaria, *P. berghei* , Piperaquine, Parasite recrudescence, Rifampicin

INTRODUCTION

Malaria is a major public health problem occurring mostly in sub-tropical countries of the world (WHO, 2021). It is a vector-borne febrile disease that is caused by Plasmodium species (Birhanie *et al*. 2014; de Carvalho *et al*., 2021; WHO, 2021). Although malaria is preventable and treatable, it has been estimated to cause 241 million infections and 627,000 deaths in 2020, especially among children below the age of 5 years (Malaria Consortium, 2020; UNICEF, 2020; WHO, 2020). Artemisinin-based combination therapy (ACT) is the most effective treatment for uncomplicated *P. falciparum* malaria in countries of WHO African endemic region (WHO, 2019). However, evidence of a widespread plasmodium resistance to malaria chemotherapy including artemisinin continues to threaten the effective control of malaria in the WHO endemic regions of the world thus compromising the global efforts to eradicate the disease (Bustamante *et al*. 2012; WHO, 2015; WHO, 2020; Maiga *et al*., 2021).

Treatment failure of *P. falciparum* to artemisinin antimalarials in Great Mekong region, Southeast Asia and in Tanzania, East Africa, respectively, have been reported (WHO, 2015; Boonyalai *et al*., 2020; Owoloye *et al*., 2021). *In vitro* and *in vivo* parasite monitoring in China, Viet Nam and Tanzania have shown evidence of treatment failure such as delayed parasite clearance time, early recrudescence and high increase in IC50 values for artemisinin and its derivatives (Amaratunga *et al*. 2016; Owoloye *et al*., 2021; WHO, 2019). In addition,

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decreased sensitivity of *P. falciparum* to artemisinin and increased rates of recrudescent infections after artemisinin-based combination therapies (ACTs) have been reported on the coast of Kenya and South-West Nigeria (Bormann *et al*., 2011; Sowunmi *et al*., 2019). Given that there are no currently available alternative therapies, improving diagnosis and follow-up, rotating drug regimens, and developing novel drug combinations to mitigate malaria drug resistance are now of greater importance. Hence the need to assess the inclusion of antibiotics with antimalarial activity in malaria chemotherapy as a valuable option (Badejo *et al*. 2014).

Antibiotics such as tetracyclines, azithromycin, fluoroquinolones and rifampicin have been shown to exhibit *in vitro* and *in vivo* antimalarial activities in acute falciparum malaria in human subjects (Gbotosho *et al*., 2012; Badejo *et al*. 2014; Mavoko *et al*., 2017). The combination of antibiotics which have different mechanisms of action from the antimalarial drug gives rise to superior efficacy, better safety and decreased resistance profile. However, the potential clinical usefulness of tetracyclines and fluoroquinolones is limited by their slow antimalarial action and contraindication in pregnant women and in children who are most affected by the disease (Gbotosho *et al*., 2012: Gaillard *et al*., 2015). On the other hand, azithromycin a macrolide has been combined with quinine, chloroquine or mefloquine with potentiation in antimalarial activity, but not without the challenge of difficulty in adherence to treatment regimen especially with the 5- 7 day dose of quinine (WHO, 2015; Mavoko *et al*., 2017). The combination of azithromycin with artesunate an artemisinin derivative has also been found to be antagonistic (WHO, 2015).

Rifampicin is a semi-synthetic broad-spectrum antibiotic produced by *Streptomyces mediterranei*. It is useful in the treatment of mycobacterium infections and several other bacterial infections including *Staphylococcus aureus*, *Legionella pneumophila*, Group A *Streptococcus*, *Brucella spp*, *Haemophilus influenza* and, *Neisseria meningitides* because of its excellent penetration and low side effects profile (Veseley *et al*., 1998; Diallo *et al*., 2018; Campbell *et al*., 2021). Limited *in vivo* studies have shown mutual beneficial antimalarial effects of combination of amodiaquine plus rifampicin, artesunate-amodiaquine plus rifampicin against *Plasmodium berghei* (Badejo *et al*., 2014). However, there are presently no published reports on the antimalarial effects of piperaquine plus rifampicin combinations against *P. berghei* infections. Thus efforts in this study were devoted to investigating the therapeutic efficacy of piperaquine plus rifampicin and piperaquine-dihydroartemisinin plus rifampicin *in vivo* during malaria infection in an animal model of *Plasmodium berghei.*

MATERIALS AND METHODS

Drug samples: Rifampicin (RIF) and chloroquine (CQ) were obtained from Bond Pharmaceuticals, Awe, Oyo State, Nigeria and Sigma St. Louis, MO, USA, respectively. While the Walter Reed Army Institute for Research, USA provided piperaquine (PPQ) and dihydroartemisinin (DHA). Stock solutions of the drugs were prepared in distilled water and stored at 4°C till required.

Animals: Swiss albino mice (6–8 weeks old) weighing 18–22 grams used in this study were obtained from the animal house of the Malaria Research Laboratories, Institute for 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.

Anti-malarial test *in vivo*: A modification of the Peters' four days suppressive test *in vivo* (Peter, 1967; Gbotosho *et al*., 2012) was used. Fifty (50) Swiss albino mice were infected intravenously with 1 x 10^7 chloroquine-resistant ANKA strain of *P. berghei* and subsequently divided into ten (10) groups randomly (n=5). Groups I-VIII animals were treated accordingly. Each animal received rifampicin (15 mg/kg body weight or 30 mg/kg body weight), piperaquine alone (16 mg/kg body weight), piperaquine (16 mg/kg body weight) plus rifampicin (15 mg/kg body weight or 30 mg/kg body weight), piperaquine (16 mg/kg body weight) plus dihydroartemisinin (2 mg/kg body weight) or piperaquine (16 mg/kg body weight) plus dihydroartemisinin (2 mg/kg body weight) plus rifampicin (15 mg/kg body weight or 30 mg/kg body weight), respectively. All doses were administered daily for three consecutive days. Group IX animals were treated with chloroquine 10 mg/kg body weight given daily for 3 days, while the animals in group X served as the negative control and received no drug treatment.

Microscopic evaluation of antimalarial activity: Blood smears were made on clean microscope slides from tail snip, air dried, fixed with methanol and stained with 10% Giemsa. Subsequently, parasitaemia in 1,000 erythrocytes was determined microscopically. The antimalarial activities of the different combinations under investigation were assessed daily from day 1 post-infection till day 14, and then on days 21, 28, 35 and 42 respectively. The percentage parasitemia was calculated using the expression;

$$\%\ parasitaemia = \frac{Number\ of\ parasitized\ red\ blood\ cells}{Total\ number\ of\ red\ blood\ cell\ count}\ x\ 100$$

The mortality of experimental animals was monitored daily, until day 42 post-infection. Chemosuppression of parasite growth in drug-treated groups were determined relative to parasite growth in the negative control group using the formula;

$$\%\ Suppression\ of\ parasite\ growth = \frac{Mean\ parasitaemia\ of\ negaitive\ control - mean\ parasitemia\ of\ \ treatment\ group}{Mean\ parasitaemia\ of\ negative\ control} * 100$$

The antimalarial activities of all the drugs and the combinations under investigation were tested in three independent experiments.

Statistical analysis: Descriptive statistics was used to determine the means, while Chi-square was used to analyse the differences in mean percentage survival rate on days following initiation of treatment. The analysis of variance (ANOVA) was used to compare differences in mean percentage of parasite growth in the different treatment groups

RESULTS

Comparative antimalarial activities of PPQ, PPQ plus DHA, PPQ plus RIF (15 mg/kg or 30 mg/kg), PPQ plus DHA plus RIF (15 mg/kg or 30 mg/kg) combinations: Parasitemia in the control animals increased from 12.9% on day 4 to peak at 45.1% on day-11 (Table 1). All animals in the

control group died by day 12. Percentage chemosuppression of parasitemia post-infection in the various treatment groups is shown in Table 1. There was marginal or no suppression of day-4 parasitemia in animals treated with varying doses of rifampicin alone (RIF 15 mg/kg, 9.9% or RIF 30 mg/kg, 0.0%) compared to control group of animals. Day 4 chemosuppression in the chloroquine-treated group of animals was 97.9%. Furthermore, in the group of animals that received PPQ alone, PPQ/RIF (15 or 30 mg/kg) or, PPQ/DHA/RIF (15 or 30 mg/kg), parasitemia on day 4 was significantly suppressed by 100% in a manner similar to PPQ/DHA, ($p>0.05$). Parasitemia in these groups of animals remained suppressed by 100% till 11 days post-infection (Table 1).

Table 1:
Chemosuppression of parasitemia by PPQ, PPQ/RIF, PPQ/DHA/RIF and PPQ/DHA in chloroquine-resistant *P. berghei* (ANKA) infected mice

Treatment group	Chemosuppression on Day 4 (%)	Chemosuppression on Day 11 (%)
Control	NA	NA
CQ	97.9	69.7
RIF 15	9.9	0
RIF 30	0	33.8
PPQ	100	100
PPQ/RIF 15	100	100
PPQ/RIF 30	100	100
PPQ/DHA	100	100
PPQ/DHA/RIF 15	100	100
PPQ/DHA/RIF 30	100	100

CQ= chloroquine, RIF= rifampicin (15 mg/kg or 30 mg/kg), PPQ= piperaquine, PPQ/RIF= piperaquine + rifampicin (15 mg/kg or 30 mg/kg), PPQ/DHA= piperaquine + dihydroartemisinin and PPQ/DHA/RIF= piperaquine + dihydroartemisinin + rifampicin (15 mg/kg or 30 mg/kg), NA= not applicable

Parasite clearance time and parasite recrudescence of infection post treatment with RIF (15 mg/kg or 30 mg/kg) alone, PPQ/RIF (15 mg/kg or 30 mg/kg), PPQ/DHA and PPQ/DHA/RIF (15 mg/kg or 30 mg/kg) in chloroquine-resistant *P. berghei* (ANKA) induced malaria in mice:

The parasite clearance time (PCT) in the group of animals that received PPQ alone, PPQ/DHA or rifampicin-based combinations was 1±0.00 day, while PCT in the group of animals treated with chloroquine was longer (2.0±0.50 days), but not significantly different ($p>0.05$). In contrast, there was no complete parasite clearance in the group of animals treated with RIF 15 or 30 mg/kg, Table 2.
Parasite recrudescence time (PRT) in treated animals after an initial parasite clearance was between 5 days and 33 days (Table 2). Parasite recrudescence time in group of animals treated with chloroquine (CQ) was 5 days, and was significantly lower ($p<0.05$) than PPQ alone and all PPQ combinations. The PRT in groups of animals that received PPQ/RIF 15 mg/kg, PPQ/RIF 30 mg/kg PPQ/DHA/RIF 15 mg/kg and PPQ/DHA/RIF 30 mg/kg were longer, but not significantly different ($p>0.05$) compared with PPQ/DHA. However, the PRT in the group of animals that received PPQ monotherapy, 17.3±3.75 days was significantly shorter ($p<0.05$) than in all the combination-treated animals.

Table 2:
Mean parasite clearance time and mean parasite recrudescence time in mice treated with PPQ, PPQ/RIF, PPQ/DHA/RIF and PPQ/DHA infected with chloroquine-resistant *P. berghei* (ANKA) strain

TREATMENT	PCT (Post-treatment)	PRT (Post-treatment)
Control	NC	NA
CQ	2.0 ± 0.50	4.6 ± 0.40
RIF15	NC	NA
RIF30	NC	NA
PPQ	1.0 ± 0.00	17.3 ± 3.75
PPQ/RIF15	1.0 ± 0.00	29.6 ± 7.59
PPQ/RIF30	1.0 ± 0.00	32.4 ± 6.28
PPQ/DHA	1.0 ± 0.00	26.2 ± 6.94
PPQ/DHA/RIF15	1.0 ± 0.00	30.4 ± 5.82
PPQ/DHA /RIF30	1.0 ± 0.00	33.0 ± 5.14

Data is presented as ± standard error of the means, PCT= parasite clearance time, PRT= parasite recrudescence time, CQ= chloroquine, RIF= rifampicin (15 mg/kg or 30 mg/kg), PPQ= piperaquine, PPQ/RIF= piperaquine + rifampicin (15 mg/kg or 30 mg/kg), PPQ/DHA= piperaquine + dihydroartemisinin and PPQ/DHA/RIF= piperaquine + dihydroartemisinin + rifampicin (15 mg/kg or 30 mg/kg), NC= no parasite clearance, NA= not applicable

Survival rate of animals: The survival rate of animals following treatment with CQ, RIF alone, PPQ alone, PPQ/DHA and rifampicin-based combinations are presented in Figure 3. The survival rate on day 14 was 100% in the group of animals that received PPQ monotherapy or, PPQ combinations. The day-14 survival rate in the group of mice that received CQ, RIF 15 mg/kg or RIF 30 mg/kg were 70%, 50%, and 40 %, respectively. All animals in the control group died before day-14. Day-42 survival rate of animals that received PPQ/RIF 15 mg/kg or PPQ/RIF 30 mg/kg was 75%, while animals that received PPQ/DHA, PPQ/DHA/RIF 15 mg/kg or PPQ/DHA/RIF 30 mg/kg had a survival rate of 67%.

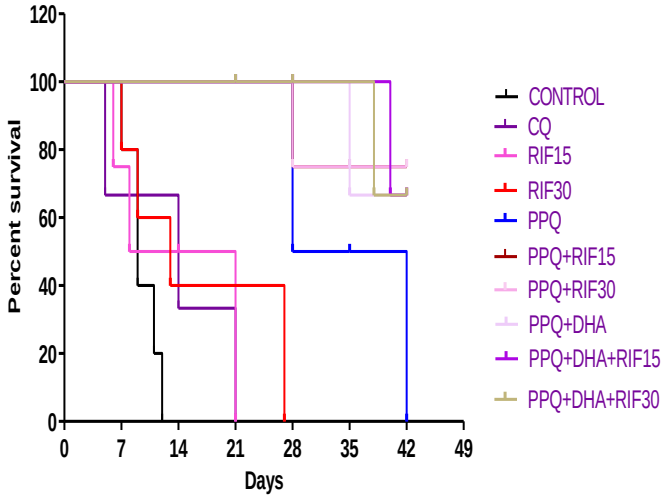

Fig. 3: Survival rate of animals infected with CQ-resistant *P. berghei*. CQ= chloroquine, PPQ= piperaquine, RIF= rifampicin (15 mg/kg or 30 mg/kg), PPQ/RIF= piperaquine + rifampicin (15 mg/kg or 30 mg/kg), PPQ/DHA= piperaquine + dihydroartemisinin, and PPQ/DHA/RIF= piperaquine + dihydroartemisinin + rifampicin (15 mg/kg or 30 mg/kg)

DISCUSSION

One of the top priorities of WHO's global strategy to defeating malaria is continuous development of antimalarial prophylaxis

and treatments which would mitigate the growing challenge of artemisinin resistant falciparum malaria (Amaratunga *et al*. 2016; WHO, 2020; Gbotosho *et al*. 2009). The malaria parasite, *P. berghei* used for this study possesses genomic sequences that are similar to those of *P. falciparum* and causes clinical features in animals that are similar to human falciparum malaria (Otto *et al*., 2014; Basir *et al*., 2012). Therefore, this model provides valuable information on the potential clinical application of antibiotic-antimalarial combination in malaria chemotherapy.

This study describes the enhancement of antimalarial activity of piperaquine or piperaquine plus dihydroartemisinin by rifampicin in mice infected with chloroquine-resistant ANKA strain of *P. berghei* . The outcome of this study is similar to the findings of Pukrittayakamee *et al*. (1994) who reported that rifampicin enhanced antimalarial activity of primaquine against *P. vivax* infection in humans. In that report, rifampicin given at the standard doses alone was active against the blood stage infection of human *P. vivax* malaria, decreasing but not clearing parasitemia. Similarly, in this study, rifampicin alone decreased *P. berghei* parasitemia in mice at the lowest tested dose, but not at the maximum dose. However, rifampicin at the tested doses when co-administered with piperaquine or piperaquine plus dihydroartemisinin exhibited good antimalarial activity against *P. berghei* malaria in mice. The findings from this study suggest potentiating effect of antimalarial activity of rifampicin when administered concomitantly with piperaquine or piperaquine plus dihydroartemisinin.

Rifampicin is an inducer of many enzymes of the cytochrome P450 super family including CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4, CYP3A5, CYP3A7 which might pose a challenge when co-administered with other drugs (Michelle *et al*., 2012; Sriniva, 2016). A previous study by Lamorde *et al*. (2013) showed significant (83%) reduction in maximum plasma concentrations of artemether, dihydroartemisinin and lumefantrine when co-administered with rifampicin-based antitubercular drug in healthy adults. It was therefore expected that the inclusion of rifampicin with PPQ or PPQ plus DHA (primarily metabolised by CYP3A4 and CYP2B6, respectively) in combinations would prolong parasite clearance time (PCT) and shorten parasite recrudescence time. The consequence of this phenomenon would be ineffective treatment. Surprisingly, the rifampicin with PPQ or PPQ/DHA combination, rather produced PCT equal to that of PPQ/DHA and within the limit of PCT acceptable for effective antimalaria drugs (WHO, 2015). The data generated in this study does not suggest induction of hepatic glucuronidation pathways which increases the metabolism of dihydroartemisinin as reported by Lamorde *et al*. (2013). Despite the concomitant administration of RIF and PPQ or PPQ plus DHA, the short treatment course of three days might have been inadequate to observe increased systemic metabolism of DHA or its partner drug, PPQ by rifampicin. This implies that the inclusion of rifampicin with PPQ or PPQ/DHA combination might be an effective alternative combination to mitigate resistance against the PPQ/DHA if deployed in malaria endemic regions.

Recrudescence is caused by incomplete clearance of parasitemia after treatment with antimalarial drugs and leads to recurrence of asexual parasitemia that caused the original illness (WHO, 2015). One major feature of recrudescence is inadequate or ineffective treatment, and should be differentiated from re-infection. In this study, after early parasite clearance, late parasite recrudescence was observed in PPQ monotherapy and the PPQ combination groups including PPQ/DHA suggestive of incomplete parasite clearance, treatment failure or perhaps, resistance. The combinations of RIF/PPQ exhibited longer and superior parasite recrudescence time compared to PPQ/DHA, however, observed values were not significantly different. This buttresses the need for strengthening the existing antimalarial combination therapy or development of newer and effective treatment options for malaria parasite infection to decrease the rapid progression of antimalaria drug resistance. Riegel and Roepe (2020) reported that resistance to PPQ by chloroquine-resistant *P. falciparum* could be mediated by mutations in *P. falciparum* chloroquine-resistance transporter (pfcrt) gene. Meanwhile, findings by Boonyalai *et al*. (2020) showed that the high rates of PPQ/DHA treatment failures for *P. falciparum* infections in Cambodia is associated with PPQ resistance. The shorter parasite recrudescence time in PPQ monotherapy relative to PPQ combination groups in mice infected with chloroquine-resistant *P. berghei* may be indicative of PPQ parasite resistance phenomenon occurring earlier in the absence of a partner drug, RIF or DHA or both.

The antibiotics azithromycin, ciprofloxacin, tetracycline, clindamycin and co-trimoxazole have been reported to exhibit good antimalarial activities when co-administered with ACTs or quinine in the treatment of *P. falciparum* malaria (Gaillard *et al*., 2015; Pessanha de Carvalho *et al*., 2021: Fontinha *et al*., 2021). Similarly, rifampicin, just like these antibiotics exhibited a more superior antimalarial activity when co-administered with PPQ or PPQ/DHA and used against *P. berghei* induced-infection in mice relative to the standard antimalarial PPQ/DHA. Although the exact mechanism behind this activity is not fully understood, however, rifampicin may be enhancing the antimalarial activity of both the artemisinin and the partner drug by the inhibition of plasmodium circular DNA that encodes for the β subunit of DNA polymerase as suggested by Pukrittayakamee *et al*. (1994) and Gardner *et al*. (1991).

CONCLUSION

This study reveals that the antimalarial activity of piperaquine or piperaquine/dihydroartemisinin against chloroquine-resistant ANKA strain of *P. berghei* is potentiated when co-administered with the anti-tubercular drug, rifampicin. The combination of rifampicin/piperaquine/dihydroartemisinin or rifampicin/piperaquine may be a potentially useful chemotherapeutic alternative in the management of malaria in endemic regions. Detailed pharmacokinetic and toxicological studies on the rifampicin-based combination drugs are necessary prior to the clinical application of the combinations.

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