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Antiplasmodial activity of rifampicin/ sulfadoxine/pyrimethamine combination on *Plasmodium berghei* infected mice

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Abstract

Introduction: The enormous expense of new medication production and the issue of multidrug opposition by plasmodium parasites has necessitated the exploration on drugs with potential for repurposing. In this study, the antimalarial properties of clinical doses of rifampicin administered with sulfadoxine/pyrimethamine was assessed in *Plasmodium berghei* contaminated mice.

Materials and Methods: Adult mice (25-30 g) were parasitized with *Plasmodium berghei*, grouped and treated per oral (p.o) with RIF, SP, and RIF/SP daily. The negative control (NC) and the positive control (PC) were treated daily p.o with normal saline (0.2 mL) and chloroquine (CQ) (10 mg/kg). The 3rd group of mice received 21.4/1.07 mg/kg body weight of sulfadoxine/pyrimethamine, while the 4th and 5th groups were administered 15 mg/kg body weight of rifampicin and 15/21.4/1.07 mg/kg body weight of rifampicin/sulfadoxine/pyrimethamine combination, respectively. Groups I and II received the stated treatments for 3 consecutive days, while group III received a single dose of treatment. Group IV received treatment for 7 consecutive days while group V received a single dose of sulfadoxine/pyrimethamine and the specified dose of rifampicin for 7 consecutive days.

Results: There was significant reduction in parasitemia in all the studies (prophylactic, suppressive, and curative) at the drug doses used: SP (21.4/1.07 mg/kg) ($p<0.001$), RIF (15mg/kg) ($p<0.01$), RIF/SP (15/21.4/1.07 mg/kg) ($p<0.0001$) when compared to the negative control. There was an approximately two and half-fold decrease in percentage parasitemia in the rifampicin/sulfadoxine/pyrimethamine treatment group when compared to the chloroquine treatment group. By the 7th day post-inoculation, Percentage parasitemia in the rifampicin/sulfadoxine/pyrimethamine combination group had reduced to 0.68%.

Conclusion: RIF may be repurposed in combination with SP for malaria treatment.

Keywords: *Plasmodium berghei*; Rifampicin; sulfadoxine; pyrimethamine; Wistar, repurposing

1. Introduction

Malaria remains one of the greatest health concerns of people in the developing world, affecting almost half of the world's population, and defying attempts at eradication owing to the rapid development of resistance to available therapeutic agents by both the plasmodium parasite and its vector, the female anopheles mosquito. Artemisinin based Combination which has been the recommended protocol of malaria chemotherapy is beginning to grapple with the challenge of emerging resistance in Africa and Asia [1].

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In artemisinin-resistant infections the residuum of parasites not cleared before the elimination of the drug from circulation increases by several orders of magnitude leading to higher failure rates and partner drug resistance is selected. A promising approach to address this challenge has been the proposed use of two slowly eliminated partner drugs combined with an artemisinin derivative, the so-called triple artemisinin-containing combination anti-malarial treatments (TACTs). Regimens combining three anti-malarials are not entirely new, but have usually not utilized an artemisinin backbone, and include the QAP regimen of quinine–atebrine–plasmoquine which was widely used before and during the Second World War, and MSP (mefloquine–sulfadoxine–pyrimethamine) promoted in Thailand in the 1980s [2].

The challenges posed by emerging artemisinin-resistant malaria and the opportunities in drug repurposing have prompted this research into the use of an antibiotic combination with other drugs with established antimalarial potency to control the malaria burden in resource poor settings.

Rifampicin is a macrocyclic antibiotic characterized by a chromophoric naphthohydroquinone group that is spanned by a long aliphatic bridge, with an acetyl group at C25. Although there are no clinical trials against malaria in humans, rifampicin has been known to have activity in vitro against all erythrocytic stages of P. falciparum [3, 4]. Its mechanism of action by which it inhibits Plasmodium parasite could be likened to the way it exhibits its bactericidal effect on bacteria since the Plasmodium parasites contain an organellar circular DNA molecule that encodes the beta subunit of a prokaryocyte-like RNA polymerase [5]. In bacteria, rifampin inhibits DNA-dependent RNA synthesis through its effect on the beta subunit of eubacterial RNA polymerase [6]. This study assessed the antimalarial activity of Rifampicin (RIF) and its combination with Sulfadoxine/Pyrimethamine (SP) in the treatment of P.berghei infected mice.

2. Material and methods

2.1. Experimental Animals and Malaria Parasite

Wistar mice weighing 25- 30 g were used. They were purchased from the Animal House of the Department of Pharmacology, University of Port-Harcourt, Nigeria. The mice were kept in cages and allowed to acclimatize for 2 weeks before the study began. The mice were fed with food and water ad libitum. *P. berghei* was acquired from the Malaria Research Laboratory, Centre for Malaria Research and Phytomedicine, University of Port-Harcourt, Nigeria, and used to prepare stock inoculum of 1 x 10^7 *P. berghei* infected erythrocytes in 0.2 mL by diluting rations of the blood infected with *P. berghei* with 0.9% normal saline.

2.2. Drugs

Sulfadoxine/pyrimethamine (SP), Rifampicin (RIF), and chloroquine (CQ) were gotten by JOSIVIC MEDICALS LIMITED, Okuru-Abuloma road, Port Harcourt, Rivers State, Nigeria. Stock solutions of the compounds were prepared in distilled water and stored at −20 °C till required.

2.3. Anti-Malarial Tests *in vivo*

Standard procedures which involve the inoculation of male Swiss Mice with *P. berghei* and subjecting small groups of the inoculated mice to various treatments including rifampicin as monotherapy, and rifampicin/sulfadoxine/pyrimethamine combination, was conducted. All compounds and combinations were tested in three independent experiments.

2.3.1. Curative Test

The method of Ryley and Peters method (1970) was adopted. Thirty (30) mice were randomly assigned into groups of five, giving rise to 6 independent groups labelled groups I to VI. The first group was designated as normal control (non-parasitized), while the second group was designated as the negative control. All groups of mice, except for the first group (normal control), were inoculated with 1 x 10^7 *P. berghei* parasitized erythrocytes intraperitoneally (IP). Various treatments were then applied to mice in all the groups.

72 hours (3 days) after the inoculation with established infection, the following treatments were applied to the various groups. Mice in Groups I and II were treated with normal saline (0.2 mL), while mice in Group III designated as the positive control, were treated with 10 mg/kg body weight of chloroquine (CQ). Mice in Group IV, were treated with 21.4/1.07 mg/kg body weight of sulfadoxine/pyrimethamine combination. Similarly, mice in group V received 15 mg/kg body weight of Rifampicin. Group VI received 15/21.4/1.07 mg/kg body weight of rifampicin/dihydroartemisinin/piperaquine combination. Groups I, II, III and IV received the stated treatments for 3

consecutive days, while group V received the specified dose of rifampicin for 7 consecutive days. a single dose of treatment. Group VI received a single dose of sulfadoxine/pyrimethamine and the specified dose of rifampicin for 7 consecutive days.

Parasiticidal activity was assessed daily from day 3 post-infection till day 14. Tail blood smears were prepared, methanol-fixed, stained with Giemsa and microscopically examined to determine parasite density.

2.3.2. Suppressive Test

Suppressive testing was conducted after the method reported by Knight and Peters (1980). Twenty-five (25) mice were randomly assigned into groups of five, giving rise to five independent groups labelled groups I to V. The first group was designated as negative control (non-parasitized), while the second group was designated as the positive control. Mice in all groups were inoculated with 1 x 10^7 *P. berghei* parasitized erythrocytes intraperitoneally (IP). Three hours after the inoculation, the following treatments were applied to the various groups. Mice in Group I were treated with normal saline (0.2 mL), while mice in Group II designated as the positive control, were treated with 10 mg/kg body weight of chloroquine (CQ). Group III received 21.4/1.07 mg/kg body weight of sulfadoxine/pyrimethamine combination, while group IV were administered 15 mg/kg body weight of Rifampicin. Group V received 15/21.4/1.07 mg/kg body weight of rifampicin/sulfadoxine/pyrimethamine combination.

Groups I and II received the stated treatments for 3 consecutive days, while group III received a single dose of treatment. Group IV received treatment for 7 consecutive days while group V received a single dose of sulfadoxine/pyrimethamine and the specified dose of rifampicin for 7 consecutive days.

Parasiticidal activity was assessed daily from day 3 post-infection till day 14. Tail blood smears were prepared, methanol-fixed, stained with Giemsa and microscopically examined to determine parasite density.

2.3.3. Prophylactic test

Prophylactic testing was done by first randomly grouping the mice into five treatment groups similar to the suppressive testing previously described. However, treatment was commenced in all the groups, 4 days prior to the inoculation of the mice with *P. berghei*. On the day of inoculation (day 4), mice in all the treatment groups were injected intraperitoneally with *P. berghei* parasitized erythrocytes and treatment continued for 3 days in groups I – II, while group III received a single dose of treatment post-inoculation. Group IV received treatment for 7 consecutive days and group V received a single dose of sulfadoxine/pyrimethamine and the specified dose of rifampicin for 7 consecutive days, post-inoculation.

Percentage parasitemia was determined using the formula below:

$$\% \text{ parasitemia} = \frac{total\ number\ of\ parasitized\ RBC}{total\ number\ of\ RBC} \text{ x } 100$$

$$\% \text{ inhibition} = \frac{(mean\ parasitemia\ of\ negative\ control - mean\ parasitemia\ of\ treated\ groups)}{mean\ parasitemia\ of\ negative\ control} \text{ x } 100$$

2.4. Mean Survival Time

The average survival time in days, of the mice in each treatment group was determined by observing and documenting mortality in each group then applying the formula:

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

2.5. Determination of Hematological and Lipid profile parameters

Blood samples of mice used for the curative test were collected and evaluated to determine their RBC, HB, PCV, WBC, and platelet counts. Cholesterol, triglyceride and HDL-cholesterol analyses were performed.

2.6. Data analysis

Data analysis was done using IBM SPSS statistics (2017) software. Data was presented as mean ± standard error of the mean (SEM). Significant difference was evaluated through the one-way analysis of variance (ANOVA). Significance was considered at $p<0.05$; $p<0.01$ and $p<0.001$.

3. Results

3.1. Curative test

The reduction in parasitemia percentage in mice treated with sulfadoxine/pyrimethamine was significant in comparison to negative control, at $p<0.001$. There was also a significant reduction in percentage parasitemia when compared to negative control, in the group treated with rifampicin and its combination with sulfadoxine/pyrimethamine, at $p<0.0001$. This reduction in percentage parasitemia was even more statistically significant than the reduction of parasitemia in the groups treated with either chloroquine or rifampicin. There was a statistically significant difference ($p<0.05$) in the decrease in parasitemia between the groups treated with chloroquine and those treated with rifampicin/sulfadoxine/pyrimethamine combinations (table 1).

There was an approximately two and a half-fold decrease in percentage parasitemia in the rifampicin/sulfadoxine/pyrimethamine treatment group. By the 7th day post-inoculation, Percentage parasitemia in the rifampicin/sulfadoxine/pyrimethamine combination group had declined to 0.68%. This decline in percentage parasitemia translated to a percentage parasitemia inhibition of 96.89%.

Table 1 Curative activity of rifampicin & combination with SP on *P. berghei*-infected mice

Treatment	Parasitemia %	MST (days)	Inhibition (%)
NC	21.91±0.51	7.60±3.50	-
CQ	1.70±0.21^{a}	28.8±2.50	92.24
SP	1.87±0.19^{a}	29.0±2.00	91.47
RIF	0.87±0.17^{b}	30.0±2.50	96.03
RIF/SP	0.68±0.26$^{c, d}$	32.4±2.50	96.89

KEY: Nc – Negative control; CQ – Chloroquine; SP – Sulfadoxine/pyrimethamine; RIF – Rifampicin; RIF/SP – Rifampicin/Sulfadoxine/pyrimethamine; MST – mean survival time; Data is expressed as mean ± standard error of the mean. a $p<0.001$ when compared to NC; bp<0.01 when compared to NC; cp<0.0001 when compared to NC; dp<0.05 when compared to CQ.

3.2. Suppressive test

The findings from the suppressive test closely match those of the curative test in terms of significant reduction in percentage parasitemia and percentage inhibition of the *Plasmodium berghei* parasite by the individual doses of the drugs used in the study (except the negative control) as well as the respective combinations with rifampicin (table 2).

Table 2 Suppressive activity of rifampicin & combination with SP on *P. berghei*-infected mice

Treatment	Parasitemia %	MST (days)	Inhibition (%)
NC	23.93±0.49	8.40±2.0	-
CQ	1.35±0.25^{a}	29.40±3.00	94.36
SP	1.11±0.22^{a}	31.20±1.50	95.36
RIF	0.56±0.18^{b}	32.80±2.00	97.66
RIF/SP	0.55±0.23$^{c, d}$	33.00±3.00	97.70

KEY: Nc – Negative control; CQ – Chloroquine; SP – Sulfadoxine/pyrimethamine; RIF – Rifampicin; RIF/SP – Rifampicin/Sulfadoxine/pyrimethamine; MST – mean survival time; Data is expressed as mean ± standard error of the mean. a $p<0.001$ when compared to NC; bp<0.01 when compared to NC; cp<0.0001 when compared to NC; dp<0.05 when compared to CQ.

Percentage parasitemia averaged 23.93% on day 7 post-inoculation in the negative control group. At the same time, Percentage parasitemia in the rifampicin/sulfadoxine/pyrimethamine combination group had declined to 0.55% indicating a percentage parasitemia inhibition of 97.70%.

3.3. Prophylactic test

As observed with the curative and suppressive tests, the various treatments (except the negative control) led to significant reductions in parasitemia ($p<0.001$). More than three-fold reduction in percentage parasitemia in the rifampicin/sulfadoxine/pyrimethamine treatment group was observed when compared to the positive control group (chloroquine treatment group). After seven days post-inoculation, parasitemia had declined to 0.47% in the rifampicin/sulfadoxine/pyrimethamine combination group (table 3). The percentage inhibition of the parasites across the treatment groups ranged from 94.33% to 99.86%, with sulfadoxine/pyrimethamine treatment group having the least parasite inhibition. Average survival time of the mice was greatest in the rifampicin/sulfadoxine/pyrimethamine combination group.

Table 3 Prophylactic activity of rifampicin & its combination with SP on *P. berghei*-infected mice

Treatment	Parasitemia %	MST (days)	Inhibition (%)
NC	21.02±0.89	8.20±4.00	-
CQ	1.57±0.21[a]	26.40±2.00	92.53
SP	1.05±0.46[a]	25.20±1.50	95.01
RIF	0.60±0.44[b]	25.60±3.00	97.15
RIF/SP	0.47±0.49[c, d]	29.20±2.50	97.76

KEY: Nc – Negative control; CQ – Chloroquine; SP – Sulfadoxine/pyrimethamine; RIF – Rifampicin; RIF/SP – Rifampicin/Sulfadoxine/pyrimethamine; MST – mean survival time; Data is expressed as mean ± standard error of the mean. a $p<0.001$ when compared to NC; bp<0.01 when compared to NC; cp<0.0001 when compared to NC; dp<0.05 when compared to CQ.

3.4. Effects of rifampicin on lipid profile and hematological parameters

The lipid profile and hematological parameters of mice in the various treatment groups are displayed in tables 4 and 5. Remarkably, the negative control group had the highest increase in values of all the lipid profile parameters with high triglycerides (169.94±7.88 mg/dL), high total cholesterol (200.08±9.11 mg/dL), high HDL (81.96±3.01 mg/dL), and high LDL (124.59±5.78 mg/dL). In comparison, the normal control group had triglycerides (121.81±5.91 mg/dL), total cholesterol (106.03±7.95 mg/dL), HDL (56.03±3.06 mg/dL), and LDL (86.82 ± 4.99 mg/dL). In the groups which received treatment, triglycerides were lower than in the untreated group (negative control), but higher than the normal control (the group without parasite inoculation). Similarly, total cholesterol was lower than in the negative control group, but higher than in the normal control group. HDL and LDL values were variable across all treatment groups.

Table 4 Effect of rifampicin & its combination with SP on lipid profile of *P. berghei*-infected mice

Treatment	TG (mg/dL)	CHOL (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)
Nmc	122.16±6.11	106.13±9.21	55.21±2.91	87.32 ± 5.33
Nc	168.86±8.11	193.67±9.66	82.11±2.67	125.03±6.10
CQ	163.70±10.28[a]	162.67±12.03[a]	61.23±1.56[a]	134.83±7.03[a]
SP	148.24±7.96[a]	126.95±13.23[a]	60.19±1.17[a]	114.46±7.03[a]
RIF	121.17±9.24[b]	108.94±10.41[b]	53.75±1.81[b]	88.94±7.03[b]
RIF/SP	149.52±7.07[c, d]	125.18±11.08[c, d]	52.46±3.77[a]	121.94±7.03[c, d]

KEY: Nmc – normal control; Nc – Negative control; CQ – Chloroquine; SP – Sulfadoxine/pyrimethamine; RIF – Rifampicin; RIF/SP – Rifampicin/Sulfadoxine/pyrimethamine; TG –triglyceride; CHOL – Total cholesterol; HDL-C – High density lipoprotein cholesterol; LDL-C – Low density lipoprotein cholesterol.; Data is expressed as mean ± standard error of the mean. a $p<0.001$ when compared to NC; bp<0.01 when compared to NC; cp<0.0001 when compared to NC; dp<0.05 when compared to CQ

There was a general decline in the hemoglobin levels and red cell counts of all the treatment groups, as well as an increase in the white cell counts of the various treatment groups when compared to the normal control (table 5). The packed cell volume, hemoglobin level, and white blood cell counts of the negative control, chloroquine and sulfadoxine/pyrimethamine treatment groups were similar.

Table 5 Effect of rifampicin & its combination with SP on hematological profile of *P. berghei*-infected mice

Treatment	PCV (%)	Hb (g/dl)	RBC ($\times10^6$/µl)	WBC ($\times10^3$/µl)	Platelet ($\times10^3$/µl)
NmC	52.4±2.14	16.71±0.89	5.45±0.22	5.66±0.15	257.00±12.61
NC	26.0±2.09	8.67±0.54	4.00±0.15	6.33±0.37	249.00±14.12
CQ	26.0±2.16[a]	8.67±0.77[a]	3.90±0.20[a]	8.03±0.17[a]	235.00±9.16[a]
SP	23.7±3.51[a]	7.87±0.51[a]	3.60±0.17[a]	9.50±0.20[a]	258.33±7.51[a]
RIF	29.0±2.97[b]	9.67±0.76[b]	4.20±0.19[b]	5.50±0.21[b]	244.00±8.54[b]
RIF/SP	28.7±3.71[cd]	9.57±0.85[c, d]	4.37±0.20[c, d]	11.13±0.17[c, d]	247.33±13.77[c, d]

KEY: Nmc – normal control; Nc – Negative control; CQ – Chloroquine; SP – Sulfadoxine/pyrimethamine; RIF – Rifampicin; RIF/SP – Rifampicin/Sulfadoxine/pyrimethamine; PCV –packed cell volume; Hb – hemoglobin; RBC – red blood cell; WBC – white blood cell.; Data is expressed as mean ± standard error of the mean. a $p<0.001$ when compared to NC; b$p<0.01$ when compared to NC; c$p<0.0001$ when compared to NC; d$p<0.05$ when compared to CQ

4. Discussion

Drug discovery is a long and complex process. It is capital intensive with no assurance of success. In recent times, there has been a significant decline in the number of new drugs approved for clinical use. This has necessitated the repurposing of already approved drugs, for new indications other than that for which they were initially approved. This reduces costs and research time considerably [9]. The observed decline in parasite load in the rifampicin treatment group up to 7days post-inoculation was in agreement with the findings of Strath, et al. [4] but was contrary to the findings of Badejo et al. [10] which study showed significant difference in parasite load only up to the 3rd day post-inoculation, compared to the control group.

Anemia is a common malaria complication prevalent in children and pregnant women in malaria endemic regions [11]. *P. berghei* infected mice suffer from anemia because of erythrocyte destruction, either by parasite multiplication or by spleen reticuloendotelial cell action as the presence of many abnormal erythrocytes stimulates the spleen to produce many phagocytes [12]. The current study showed that anemia in Negative control was characterized by decreased RBC, PCV, HB with increased WBC levels. However, RIF/SP produced reduction in anemia characterized by increased RBC, PCV, HB and decreased WBC levels. RIF/SP produced the best effects on the hematological parameters than individual doses of RIF and SP. Evolving studies advise that routine laboratory measurement of lipids could be a reliable aid in the early judgment of malaria especially in places that are malaria endemic [13]. This study observed impaired lipid profile characterized by elevated TG, CHOL, and LDL-C and decreased HDL levels in negative control. This observation is consistent with altered lipid profile reported in previous findings [14]. However, lipid levels were restored in parasitized rats treated with RIF/SP. This study showed significant efficacy of rifampicin and its combinations in the clearance of plasmodium parasitemia without adversely modifying the hematological and lipid profiles of the mice used for the study.

5. Conclusion

The study shows that RIF may be repurposed in combination with SP for the treatment of malaria.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors hereby declare that no conflict of interest exists.

Statement of ethical approval

Ethical approval for this research was sought, and same was granted by the centre for research ethics and management of the university of Port Harcourt.

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