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Antiplamodial effect of sulfadoxine/pyrimethamine/clindamycin: A study in parasitized mice

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Abstract: Triple antimalarial combination therapies may overcome the emergence of antimalarial drug resistance. Sulfadoxine/pyrimethamine (S/P) is an antimalarial drug. Clindamycin (C) has potential antiplasmodial effect. This study assessed whether the antiplasmodial activity of S/P can be augmented by C on *Plasmodium berghei*-infected mice. Adult Swiss albino mice (25-30g) were grouped and infected with *Plasmodium berghei*. The mice were orally treated daily with S/P (21.4/10.7 mg/kg), C (10mg/kg) and S/P/C, respectively using curative, prophylactic and suppressive tests. The normal and negative controls were treated daily with normal saline (0.2mL) while the positive control was orally treated with chloroquine (CQ) (10mg/kg). After treatment, blood samples were collected and evaluated for percentage parasitamia and hematological parameters. Mice were observed for mean survival time. In the curative, suppressive and prophylactic tests, S/P/C significantly decreased parasitamia levels when compared to SP or C at $p< 0.05$. S/P/C significantly prolonged mean survival time when compared to S/P or C with difference at $p< 0.05$. S/P, C, and S/P/C produced 65.62 %, 62. 03 % and 85.31 % parasitamia inhibitions, respectively while CQ produced 83.72 % parasitamia inhibition. S/P/C caused significant reduction in anemia marked by increased packed cell volume, hemoglobin, red blood cells and decreased white blood cells at $p< 0.05$ when compared to SP or C. S/P/C eradicates liver merozoites and central vein congestion. C increased the antiplasmodial activity of S/P, therefore S/PC may be used for malaria treatment.

Keywords: Triple regimen, drug, combination, antimalaria, mice

1 Introduction

Plasmodium resistance, which emerged slowly after the introduction of antimalarial drug is widespread and has become a serious challenge to malaria treatment and eradication (Feachem et al., 2018; Ashley et al., 2014). *Plasmodium* resistance against sulfadoxine-pyrimethamine and chloroquine and most recently artemisinin-based combination therapies (ACTs) has been documented. This shows the need for antimalarial drug pipeline featuring compounds with novel modes of action or repurposed drugs until malaria eradication is achieved. While new compounds are been discovered, additional strategies are urgently needed to curb the persistent and rapid emergence of *Plasmodium* resistance to artemisinins and partner drugs (Mekonnen, 2015). This may involve the use of triple antimalarial combination therapies, which combine ACTs or other antimalarial drugs with partner drugs that are slowly eliminated. This may provide effective treatment and delay the emergence of *Plasmodium* resistance (Dini *et al.*, 2018; Vander Plijm *et al.*, 2020).

Sulfadoxine/Pyrimethamine (S/P) is used for malaria prophylaxis in pregnancy and malaria treatment. It is used as a partner drug with ACTs especially in Africa (WHO, 2012; 2015) to overcome *Plasmodium falciparum* resistance (Leslie *et al.*, 2017). However, there is an emergence of *Plasmodium* resistance to S/P and ACTs (Menard and Dondorp, 2017; Woodrow and White, 2017). The emergence of *Plasmodium* resistance has increased the search for novel antimalarial drugs, including partners' drugs through convectional and non-convection methods (Kremsner *et al.*, 1994).

Clindamycin (C) is a lincosamide antibiotic used for the treatment of anaerobic and gram positive bacterial infections, *Pneumocystis carinii* pneumonia, toxoplasmosis and babesiosis. C acts by inhibiting bacterial protein synthesis at the level of 50S ribosome (Smeija, 1998). Studies showed it is effective against malaria caused by *Plasmodium falciparum*, malaria. Improved effectiveness, shortened

duration of treatment and reduced risk of treatment failure were observed when it was used as a partner drug with quinine and chlroquine (Obonyo and Juma, 2012). Also, in combination with quinine it produced high malaria cure rate in mothers and children (Obonyo and Juma, 2012). This study evaluated whether C can be repurposed as a partner drug with S/P for the treatment of malaria using a mouse model infected with *Plasmodium berghei.*

2 Materials and Method

2.1 Animals, drugs and dose selection

Swiss albino mice (25–30 g) were obtained from the Animal House, of the Department of Pharmacology, Faulty of Basic Clinical Sciences, University of Port Harcourt, Nigeria. The mice were housed in plastic cages with access to chow and water *ad libitum*. The mice were acclimated for 2 weeks and handled according to the guide on animal handling by European council and the Parliament. Ethical approval for this study was provided by the Research Ethics Committee of the Department of Pharmacology/Toxicology, Faculty of Pharmacy, Niger Delta University. S/P (Artepharm Co., Ltd., China), C (Mediplantex National Pharmaceutical, Viet Nam) and CQ (Evans Pharm, Nigeria) were used. The doses used are S/P (21.4/10.7 mg/kg) (Chaponda *et al.,* 2021), C (2.2 mg/kg) (Gaillard *et al.,* 2015) and CQ (10mg/kg) (Somsak *et al.,* 2018)

2.2. Inoculation of mice with parasite

CQ sensitive *Plasmodium berghei* (NK65) was supplied in parasitized mice by the Nigerian Institute of Medical Research Yaba, Lagos. The parasite was preserved by blood passage intraperitoneally (ip) from parasitized mice to non-parasitized mice within 5–6 days of infection.

2.3. Antiplasmodial assessment of sulfadoxine/pyrimethamine/clindamycin

2.3.1. Curative test

It was performed as explained by Ryley and Peters 1980. Thirty Swiss albino mice infected i.p with 1×10^7 *Plasmodium berghei* were randomized into 6 groups of n=5/group. The normal and negative controls were treated orally with normal saline (0.2mL) while the positive control was orally treated with CQ (10mg/kg). Other groups were orally treated with S/P (21.4/10.7 mg/kg), C (2.2 mg/kg) and S/P/C, respectively. On the 5th day, tail blood samples were collected and thin blood films were prepared on slides. The slides were fixed in methanol and stained with Giemsa stain. The stained slides were viewed using a microscope and percentage parasitamia and percentage inhibitions were calculated using the formula below as reported by Adikwu and Ajeka, 2021.

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

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

2.3.2. Prophylactic test

It was performed based on the method described by Peters 1967. Thirty Swiss albino mice were grouped into 6 of n=5/group. The controls (normal and negative) were orally treated with normal saline (0.2mL) while the positive control was orally treated with CQ (10mg/kg) for 4 days. Other groups were orally treated with S/P (21.4/10.7 mg/kg), C (2.2 mg/kg) and S/P/C for 4 days, respectively. On the 5th day, the mice were inoculated i.p with blood containing $1x10^7$ *Plasmodium berghei*. After 72 hr tail blood samples were collected and percentage parasitamia and inhibitions were calculated as explained above.

2.3.3. Suppressive test

It was performed as reported by Knight and Peters 1980. Thirty mice inoculated i.p with blood containing $1x10^7$ *Plasmodium berghei* were grouped into 6 of n=5/group. After 2 hr, the mice were orally treated with S/P (21.4/10.7 mg/kg), C (2.2 mg/kg) and S/P/C daily for 4 days, respectively. Normal and negative controls were orally treated with normal saline (0.2mL) while positive control was treated with CQ (10mg/kg) orally for 4 days. On the 5th day, tail blood samples were collected, prepared and percentage parasitamia and inhibitions were calculated as explained above.

2.4. Evaluation of biochemical markers

Samples of blood collected from the group used for the curative test were evaluated for hemoglobin (Hb), white blood cells (WBCs), red blood cells (RBCs) and packed cell volume (PCV) with the aid of an auto analyzer.

2.5. Evaluation of mean survival time

The mice were observed for mortality and the mean survival time (MST) was calculated using the formula below reported by Adikwu and Ajeka, 2021.

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

2.6. Statistical analysis

Values as mean ± SEM (standard error of mean) of n=5. One-way analysis of variance (ANOVA) and Tukey's *post hoc test* were used for data analysis. Significance was set at $p< 0.05$.

3. Results

3.1.Curative effect of sulfadoxine/ pyrimethamine/ clindamycin on mice infected with *Plasmodium berghei.*

Treatment with S/P/C decreased percentage parasitamia with significant difference observed at $p<0.05$ when compared to treatment with S/P or C. S/P, C and S/P/C showed 65,62%, 62.03% and 85.31% inhibitions, respectively whereas CQ produced 83.27% inhibition (Table 1). Treatment with S/P/C significantly prolonged MST with difference observed at $p<0.05$ when compared to treatment with S/P or C (Table.1).

3.2. Prophylactic effect of sulfadoxine/ pyrimethamine/ clindamycin on mice infected with *Plasmodium berghei*

Treatment with S/P/C decreased percentage parasitamia with significant difference observed at $p<0.05$ when compared to treatment with S/P or C (Table 2). The inhibitions which represent 75.22%, 72.27%, 97.76% and 96.25 % were produced by S/P, C, S/P/C and CQ, respectively (Table.2). S/P/C prolonged MST significantly with difference observed at $p<0.05$ when compared to S/P or C (Table 2).

3.3. Suppressive effect of sulfadoxine/ pyrimethamine/ clindamycin on mice infected with *Plasmodium berghei*

S/P/C decreased percentage parasitamia significantly when compared to S/P or C with difference observed at $p<0.05$. The inhibitions produced by S/P, C and S/P/C represent 72.40%, 70.63% and 94.38%, respectively while CQ produced 93.80% inhibition (Table 3). S/P/C prolonged MST with significant difference observed at $p<0.05$ when compared to S/P or C (Table 3).

3.4. Effect of sulfadoxine/pyrimethamine/clindamycin on hematological indices of mice infected with *Plasmodium berghei*

Reduced RBCs, PCV and Hb and increased WBCs occurred significantly ($p<0.05$) in *Plasmodium bergei* infected mice (Table 4). However, treatment with S/P/C significantly increased RBCs, PCV, Hb and significantly decreased WBCs with difference observed at $p<0.05$ when compared to S/P or C (Table 4).

3.5. Effect of sulfadoxine/pyrimethamine/clindamycin on liver histology of mice infected with *Plasmodium berghei*

The liver of normal control mice showed normal histology (Figure 6a) whereas the liver of the negative control showed normal hepatocytes, congested sinusoids, central vein congestion and merozoites (Figures 6b and 6c). The liver of CQ-treated mice showed normal hepatocytes and central vein congestion (Figure 6d). The liver of C-treated mice showed central vein congestion, and merozoites (Figure 6e) while the liver of S/P treated mice (Figure 6f) and the liver of S/P/C-treated mice (Figure 6g) showed normal hepatocytes and congested Sinusoids.

Table 1. Curative effect of sulfadoxine/pyrimethamine/clindamycin on mice infected with *Plasmodium berghei.*

Treatment	% Parasitamia	% Inhibition	MST (Days)
NC	31.26±1.23	0.0	9.05±0.97
CQ	5.09±0.11[a]	83.72	27.6±3.10 [a]
S/P	10.75±0.15[b]	65.62	22.1±3.22 [b]
C	11.86±0.88[b]	62.03	20.4±2.12 [b]
S/P/C	4.59±0.02[a]	85.31	30.8±4.07 [c]

Data as mean± standard error of mean, n=5, NC: Negative control, CQ: Chloroquine (Positive control), C: Clindamycin, S/P: Sulfadoxine/pyrimethamine, MST: Mean survival time. Values with difference superscripts down the column significantly differ at $p<0.05$ (ANOVA)

Table 2. Prophylactic effect of sulfadoxine/pyrimethamine/clindamycin on mice infected with *Plasmodium berghei*

Treatment	% Parasitamia	% Inhibition	MST (Days)
NC	22.25±0.68	0.0	9.61±0.16
CQ	0.83±0.20 [a]	96.25	34.15±3.01[a]
S/P	5.51±0.01 [b]	75.22	29.86±3.40[b]
C	6.17±0.77 [b]	72.27	27.54±3.21[b]
S/P/C	0.50±0.01 [a]	97.76	37.71±5.10[a]

Data as mean± standard error of mean, n=5, NC: Negative control, CQ: Chloroquine (Positive control), C: Clindamycin, S/P: Sulfadoxine/pyrimethamine. MST: Mean survival time. Values with difference superscripts down the column significantly differ at $p<0.05$ (ANOVA: Analysis of variance)

Table 3. Suppressive effect of sulfadoxine/pyrimethamine/clindamycin on mice infected with *Plasmodium berghei*

Treatment	% Parasitamia	% Inhibition	MST (Days)
NC	27.86±2.10	0.00	9.23±0.13
CQ	1.72±0.20 [a]	93.80	30.26±3.17[a]
S/P	7.69±0.16 [b]	72.40	28.73±3.25[b]
C	8.18±0.53 [b]	70.63	25.14±3.44[b]
S/P/C	1.58±0.04 [a]	94.38	33.08±7.03[a]

Data as mean± standard error of mean, n=5, Negative control, CQ: Chloroquine (Positive control), C: Clindamycin, S/P: Sulfadoxine/pyrimethamine. MST: Mean survival time. Values with difference superscripts down the column significantly differ at $p<0.05$ (ANOVA: Analysis of variance)

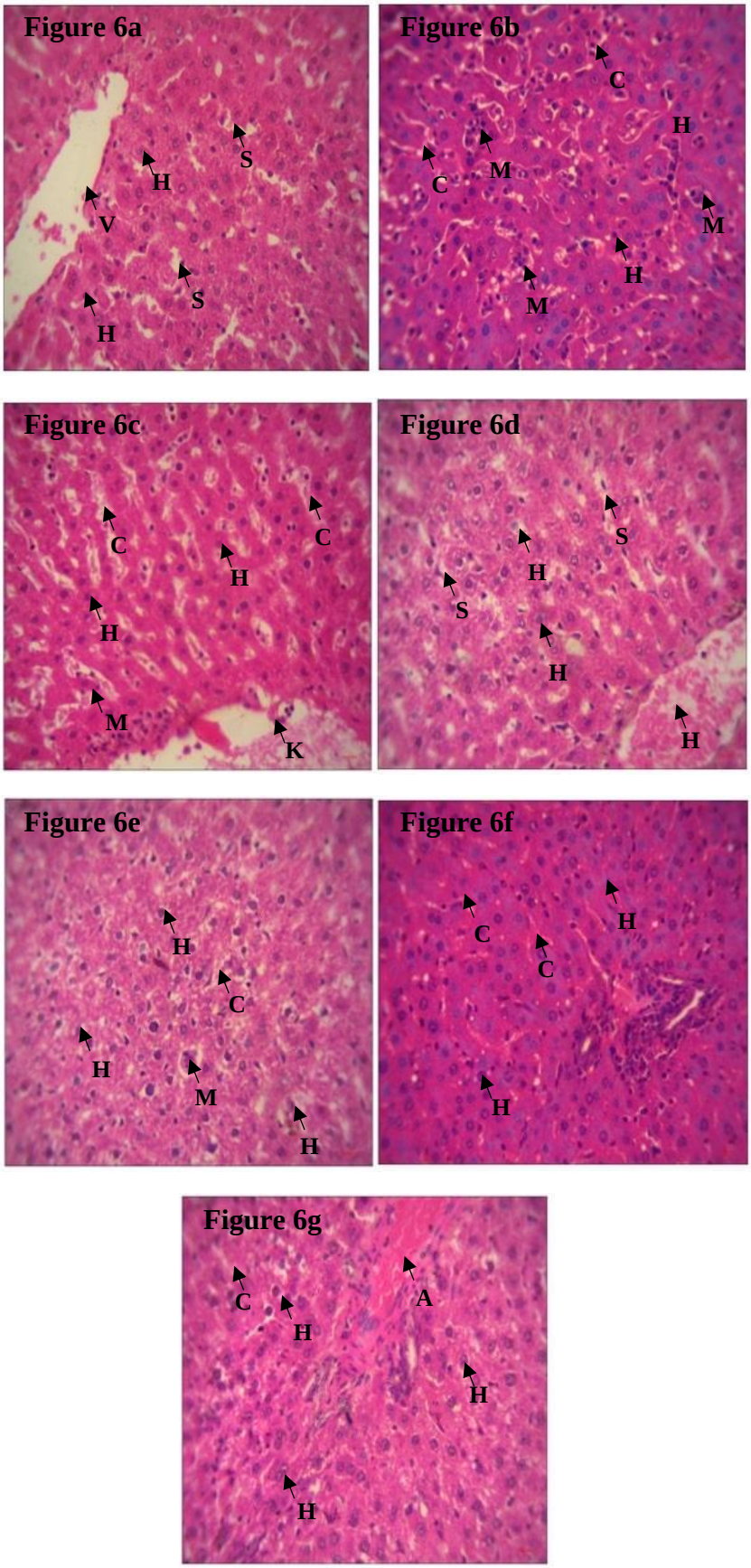
Table 4. Effect of sulfadoxine/pyrimethamine/clindamycin on hematological indices of mice infected with *Plasmodium berghei*

Treatment	RBCs ($x10^6$)	WBCs (cells/L)	PCV (%)	Hb (g/dL)
NM	6.85±0.02	4.76±0.40	58.54±5.18	15.64±0.38
NC	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]
S/P	3.35±0.15 [d]	8.77±0.36 [d]	34.74±4.98[d]	10.50±0.47[d]
C	3.10±0.27 [d]	9.63±0.52 [d]	31.17±3.55 [d]	10.01±0.31[d]
S/P/C	5.94±0.56 [c]	5.00±0.30 [c]	52.03±5.13 [c]	14.95±1.33[c]

Data as mean± standard error of mean, n=5, NM: Normal control, NC: Negative control, CQ: Chloroquine (Positive control), C: Clindamycin, S/P: Sulfadoxine/pyrimethamine. 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)

4. Discusion

Malaria is a public health problem that affects mostly people living in Sub-Sahara Africa. Children below age 5 are the most vulnerable group affected, with an estimate of 67% (247,000) of all malaria mortality worldwide (Ashley *et al.,* 2004). The increasing prevalence of drug resistant parasites now threatens the efficacies of antimalarial drugs in Sub-Saharan African (Targett *et al.,* 2001). There is currently a concerted effort to evaluate new antimalarial drug combinations through drug repurposing. The discovery of new antimalarial drug combinations can reduce post treatment parasite transmission so as to counteract the transmission of drug resistant parasites (Mekommen. 2015). The present study aim at assessing whether C can augment the antimalarial activity of S/P in a mouse model infected with *Plasmodium berghei*. A 4 day curative test was used to evaluate the antiplasmodial activity of the drug combination on establishment infection whereas suppressive test was used to determine the antiplasmodial activity on early infection (Fidock *et al.,* 2014). Mouse model was used for this study, because it allows the investigation of long term immunity to *Plasmodium* parasites, disease progress and permits studies of organs to which the parasite sequesters, such as the spleen and liver which is difficult in humans (Wykes, 2009). Many of the antimalarial drugs used currently emerged from small molecules, whose antimalarial activities were assessed in animal models (Peter *et al.,* 1998). *Plasmodium berghei* was used for this study, because of its ability to sequester within the micro-circulation, which is the characteristic of severe malaria especially the cerebral form. [7] In this study, S/P/C decreased percentage parasitamia in the curative and suppressive tests with similar effect as the standard (CQ). Also, in the prophylactic test, S/PC decreased percentage parasitamia with similar effect as CQ. In addition to the antiplasmodial assessment of S/P/C, the current study further assessed its impact on MST. In the curative, prophylactic and suppressive tests, S/P/C prolonged MST most than its constituent drugs. Also, the prolongation of MST by S/P/C was at par with CQ. Anemia, a common complication of malaria is a consequence of the hemolysis of infected and uninfected erythrocytes and bone marrow dyserythropoiesis by *Plasmodium* parasites (White, 2018). This study observed anemic signs in *Plasmodium berghei* infected mice characterized by altered levels of hematologic indices which support earlier reports (Georgewwill *et al.,* 2021). However, treatment with S/P/C curtailed the anemic impact of *Plasmodium berghei*. The colonization of the liver by *Plasmodium* parasites is an integral part of malaria infection. After an infectious mosquito bite, sporozoites find their way to hepatocytes, where liver stage development occurs. A single infectious mosquito bite can lead to liver infection, which sets the stage for successful host colonization by *Plasmodium* parasites (Vaughan and Kappe, 2017).



Figures 6a-6g. Liver micrographs of the control and experimental mice. Figure 6a: control mice, Figures 6b and 6c: Parasitized mice. Figure 6d: Treatment with chloroquine. Figure 6e: Treatment with clindamycin. Figure 6f: Treatment with sulfadoxine/pyrimethamine. Figure 6g: Treatment with sulfadoxine/pyrimethamine/clindamycin. V: Central vein K: Central vein congestion, M: Merozoites, S: Sinusoids, C: Congested sinusoids, H: Normal hepatocytes. A: Hepatic artery. X 400 H&E

This makes it imperative for the assessment of the antiplasmodial effect of drug candidates at the liver stage of infection. In this study, congested sinusoid, merozoites and central vein congestion were observed in the liver of *Plasmodium berghei*-infected mice, which support earlier reports (Ooji, 2009; Udonkang et al., 2018). The aforementioned liver changes were eradicated in mice treated with S/P/C. This observation showed that S/P/C may cure the liver stage of *Plasmodium* infection. The observed antiplasmodial activity of S/P/C may be due to the abilities of its constituent drugs to target *Plasmodium* parasites at different sites. S/P inhibits dihydrofolate reductace and dihydropteorate synthase in *Plasmodium* parasites thereby preventing folic acid synthesis (Hayton *et al.*, 2002). C inhibits protein synthesis in bacteria via activity at 50s ribosome, but its antiplasmodial activity is attributed to the inhibition of *Plasmodium* apicoplast (Lell and Kremsner, 2002; Goodman et al., 2013).

5. Conclusion: The present study showed that C increased the antiplasmodial activity of S/PC by inhibiting blood and liver stages of *Plasmodium berghei* infection. This study suggests the use of S/P/C for the treatment of malaria.

Authors contributions. EA: study conception, design, supervision, sample collection, and data analysis, literature review and manuscript writing and editing. ISA: Design, supervision, animal handling, data analysis, literature review and manuscript writing. CON: Design, supervision, animal handling, data analysis, literature review and manuscript writing.

Conflict of interest: The authors declare no conflict of interest

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