Antiplasmodial activity of desloratadine-dihydroartemisinin-piperaquine on *Plasmodium berghei* infected mice

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**ARTICLE INFO**

*Article history:*
Received on: October 10, 2020
Accepted on: December 14, 2020
Available online: March 14, 2021

**Key words:**
Desloratadine, Dihydroartemisinin/piperaquine, malaria, Mice.

**ABSTRACT**

This study examined the antiplasmodial effect of desloratadine-dihydroartemisinin-piperaquine (DL/D/P) on *Plasmodium berghei* infected mice. Adult mice (22–25 g) were grouped, inoculated with *P. berghei*, and treated orally with DL (5 mg/kg), D/P (1.71/13.7 mg/kg), and DL/D/P daily for 4 days. The negative and positive controls were treated orally with normal saline (0.2 mL) and chloroquine (10 mg/kg), respectively, for 4 days. After treatment, blood samples were assessed for percentage parasitemia and serum biochemical parameters. Mice were also observed for mean survival time (MST). In the curative, suppressive, and prophylactic studies, DL, D/P, and DL/D/P significantly decreased percentage parasitemia at *P* < 0.001, *P* < 0.001, and *P* < 0.0001, respectively, when compared to negative control (NC). DL, D/P, and DL/D/P significantly increased MST at *P* < 0.05, *P* < 0.01, and *P* < 0.001, respectively, when compared to NC. Significant (*P* < 0.001) decreases in packed cell volume, red blood cells, hemoglobin, and high-density lipoprotein cholesterol levels with significant (*P* < 0.001) increases in total cholesterol, white blood cells, low-density lipoprotein cholesterol, and triglyceride levels were observed in NC when compared to normal control. However, the aforementioned parameters were restored by DL (P < 0.05), D/P (P < 0.01), and DL/D/P (P < 0.001) when compared to NC. DL/D/P may be an effective antimalarial drug combination.

**1. INTRODUCTION**

Globally, malaria is still a public health challenge with an estimated 405,000 deaths reported in 2018 [1]. According to the World Health Organization, Africa region still bears the largest burden of malaria morbidity, with 213 million cases (93%) reported in 2018. Children aged below 5 years are the most vulnerable group affected by malaria. In 2018, they accounted for 67% (272,000) of all malaria deaths worldwide. Malaria infection in human is caused by four species of *Plasmodium* parasite, namely, *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, and *Plasmodium malariae* [2]. *P. falciparum* is the most prevalent malaria parasite in Africa, accounting for 99.7% of estimated malaria cases in 2018. Malaria parasite infection is currently treated with artemisinin combination therapy [3]. In addition to other challenges such as cost, there is rapid development and widespread resistance to artemisinin based combination therapy in several endemic regions. This explains the need for alternate strategies for chemotherapy and chemoprophylaxis [4].

A strategy to malaria chemotherapy is to reposition, repurpose or find new uses for drugs that are indicated for other diseases. Considering the difficulties of funding antimalarial drug discovery, this strategy has the advantage of reducing cost, shortening the time of drug development as well as established safety profile [5]. It is advantageous that most existing drugs that were repurposed were safe, affordable, and available. In addition, drug repurposing has greater economic feasibility, after patents have expired. Drug repurposing has been significantly used by pharmaceutical companies for the identification of newer drugs [6]. Drug repurposing has provided novel candidates, and also drug combination regimens with artemisinin, which has increased effectiveness and decreased resistance to the artemisinin [7].

Antihistamines consist of various classes of pharmacological agents that include first generation and second generation Histamine (H1) receptor inverse agonists, which are used for the treatment of allergic and inflammatory disorders [4]. The use of antihistamines has also been proposed as preventive therapy to reduce the risk of the progression of severe malaria [8]. Encouraging results have been observed with chlorpheniramine in combination with chloroquine (CQ) to reverse resistance to CQ [9]. An early study has also demonstrated the inhibitory effects of some antihistamines on *Plasmodium* parasite [10]. Desloratadine (DL), a H1 selective receptor antagonist and active metabolite of loratadine used as an anti-allergic and an anti-inflammatory agent [11] has shown potential antimalarial activity. DL displays significant inhibitory activity against CQ-sensitive and CQ-resistant strains of *P. falciparum* [4]. Furthermore, DL exerted a marked synergistic action with CQ against CQ sensitive and resistant
parasites [4]. Hence, this study assessed the antimalarial activity of DL in combination with dihydroartemisinin-piperaquine (D/P) on *P. berghei* infected mice.

### 2. MATERIALS AND METHODS

#### 2.1. Drug, Animals, and Malaria Parasite

Adult albino mice (22–25 g) bought from the animal unit of the Department of Pharmacology, University of Port-Harcourt, Nigeria, were used. The mice were kept in cages under natural environmental conditions and allowed to acclimatize for 2 weeks before the study. The mice had free access to food and water. *P. berghei* was supplied by Malaria Research Laboratory, Centre for Malaria Research and Phytomedicine, University of Port-Harcourt, Nigeria. The directive (2010/63/EU) of the European Union Parliament and the Council on animal handling was used. CQ (Alben Healthcare Ind. Ltd.), DL (Merck & Co), and D/P (Bliss GVS Pharma Ltd. India) were used. The following doses were used; D/P (1.71/13.7 mg/kg) [12], DL (5 mg/kg) [13], and CQ (10 mg/kg) [14]. The experimental procedures were approved by the Research Ethics Committee of the University of Port Harcourt, Port Harcourt, Rivers State, Nigeria.

#### 2.2. Parasite Inoculation

Stock inoculum containing $1 \times 10^7$ *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 0.2 mL of $1 \times 10^7$ *P. berghei* was inoculated into each mouse through intraperitoneal route.

#### 2.3. Curative Test

The method described by Ryley and Peters (1970) [15] was used. Thirty mice grouped into I-VI were used. The mice in groups II-VI were inoculated with $1 \times 10^7$ *P. berghei* parasitized erythrocytes intraperitoneally (i.p). After 3 days, the mice were treated per oral (p.o) as follows: Group I normal control and Group II negative control (NC) were treated with normal saline (0.2 mL), respectively, for 4 days. Group III (positive control [PC]) was treated with CQ (10 mg/kg) for 4 days. Groups IV – V were treated with DL (5 mg/kg), D/P (1.71/13.7 mg/kg), and DL/D/P for 4 days, respectively. On each day of treatment, tail blood samples were obtained and thin blood films were produced on slides. The slides were examined under oil immersion ×100 magnification and the numbers of parasitized red blood cells (RBC) were counted against the total number of RBC in a field. Percentage parasitemia levels were calculated with the aid of the formula shown below.

#### 2.4. Suppressive Test

The method described by Knight and Peters (1980) [16] was used. Twenty five mice were parasitized i.p with erythrocytes (0.2 mL) containing $1 \times 10^7$ *P. berghei*. The mice were randomly grouped into 5 of n = 5. After 3 h, the mice were treated p.o as follows: Group I (NC) was treated with normal saline (0.2 mL) daily for 4 days. Group II (PC) was treated with CQ (10 mg/kg) daily for 4 days. Groups III – V were treated with DL (5 mg/kg), D/P (1.71/13.7 mg/kg), and DL/D/P daily for 4 days, respectively. On day 5, tail blood samples were obtained and thin films were prepared on slides. Percentage parasitemia levels were calculated using the formula below.

#### 2.5. Prophylactic Test

The method described by Peters (1965) [17] was used for prophylactic test. Twenty five mice randomized into 5 groups n = 5 were used. Group I (NC) was treated p.o with normal saline (0.2 mL) whereas Group II (PC) was treated with CQ (10 mg/kg) for 4 days. Groups III – V were treated with DL (5 mg/kg), D/P (1.71/13.7 mg/kg), and DL/D/P, respectively, for 4 days. On day 4, the mice were inoculated i.p with $1 \times 10^7$ *P. berghei* parasitized erythrocytes and treatment continued for 4 days. Tail blood samples were collected and percentage parasitemia levels determined using the formula below.

#### 2.6. Determination of Mean Survival Time (MST)

The mice in the control and the treated groups were observed for mortality and expressed in days. Mortality represented as MST was calculated using the formula below.

#### 2.7. Evaluation of Hematological and Lipid Parameters

Blood samples from the mice in the curative study were collected and assessed for packed cell volume (PCV), Red blood cells (RBC), hemoglobin (HB), white blood cells (WBC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) using an auto analyzer.

#### 2.8. Statistical Analysis

Data are presented 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 $P < 0.05$; $P < 0.01$; $P < 0.001$ and $P < 0.0001$.

### 3. RESULTS

#### 3.1. Curative Test

The curative test shows significant decreases in percentage parasitemia in mice treated with DL ($P < 0.01$), D/P ($P < 0.001$), and DL/D/P ($P < 0.0001$) when compared to NC [Table 1]. Treatments with DL, D/P, and DL/D/P produced percentage inhibitions which represent 22.5%, 33.0%, and 44.6%, respectively, on day 5. Furthermore, treatments with DL, D/P, and DL/D/P produced percentage inhibitions which represent 61.2%, 77.3% and 88.6% on day 7, respectively [Table 2]. MST was increased to 18.6 ± 1.37, 22.8 ± 3.37, and 30.9 ± 4.24 in mice treated with DL, D/P, and DL/D/P, respectively, when compared NC (9.61 ± 0.24) [Table 1].

#### 3.2. Suppressive Test

Significant decreases in percentage parasitemia were observed in mice treated with DL ($P < 0.01$), D/P ($P < 0.001$), and DL/D/P ($P < 0.0001$) when compared to NC [Table 3]. Treatments with DL, D/P, and DL/D/P produced percentage inhibitions which represent 65.3%, 73.8%, and 94.0%, respectively. MST was increased to 22.6 ± 2.37 ($P < 0.05$), 30.2 ± 2.49 ($P < 0.01$), and 35.4 ± 3.33 ($P < 0.001$) in mice treated with DL, D/P, and DL/D/P, respectively, when compared to NC (9.00 ± 0.20) [Table 3].
Percentage parasitemia were significantly decreased to 6.90 ± 0.12 (P < 0.01) 2.80 ± 0.16 (P < 0.001) and 1.10 ± 0.07 (P < 0.0001) in mice treated with DL, D/P, and DL/D/P, respectively, when compared to NC. Percentage inhibitions produced by DL, D/P, and DL/D/P represent 69.2%, 87.5%, and 95.0%, respectively [Table 4]. Furthermore, treatments with DL, D/P, and DL/D/P significantly increased MST to 23.6 ± 2.37 (P < 0.05), 30.8 ± 2.20 (P < 0.01), and 37.7 ± 3.07 (P < 0.001), when compared to NC [Table 4].

### 3.4. Effects on Hematological and Lipid Parameters

Significant (P < 0.001) decreases in RBC, HB, PCV, and HDL levels with significant (P < 0.001) increases in WBC, TG, CHOL, and LDL-C levels were observed in NC when compared to non-parasitized mice [Tables 5 and 6]. In contrast, treatment with individual doses of DL and D/P significantly increased RBC, HB, PCV and HDL levels, but significantly decreased WBC, TG, CHOL, and LDL-C at P < 0.05 and P < 0.01, respectively, when compared to NC [Tables 5 and 6]. On the other hand, treatment with DL/D/P significantly increased RBC, HB, PCV, and HDL-C levels, but significantly decreased WBC, TG, CHOL, and LDL-C levels at P < 0.001 when compared to NC [Tables 5 and 6].

### 4. DISCUSSION

The development of parasite resistance to antimalarial drugs is a major barrier to successful malaria treatment in malaria-endemic areas. It has contributed to the resurgence of malaria infection and increase in malaria associated death in recent years [18]. This challenge has encouraged the use of non-convention methods including drug repurposing to fast track the discovery of new antimalarial drugs [5]. Antihistamines are used for the treatment of allergic and inflammatory disorders [4], but emerging studies have speculated potential antimalarial activity of antihistamines including DL [8]. This study examined the antiplasmodial activity of DL in combination with D/P in mice parasitized with P. berghei. Mice model is used in experimental malaria study because it allows for detailed assessment of multiple and specific pathophysiological processes caused by malaria infection, which is not possible in humans [19]. It has been used for pragmatic antiplasmodial assessment of candidate drugs using curative, suppressive, and prophylactic methods [20]. In this study, using the curative, suppressive, and prophylactic methods, treatment with DL/D/P decreased percentage parasitemia and increased percentage inhibition best than individual doses of DL, D/P, and CQ. Malaria significantly contributes to child morbidity and mortality in the world. In 2018, sub-Saharan Africa accounts for 94% of world malaria deaths, of which 67% occurred in children under five [1]. One of the primary goals of malaria therapy is the prevention of death. For pragmatic antiplasmodial assessment of candidate drugs using curative, suppressive, and prophylactic methods, treatment with DL/D/P decreased percentage parasitemia and increased percentage inhibition best than individual doses of DL, D/P, and CQ. Malaria significantly contributes to child morbidity and mortality in the world. In 2018, sub-Saharan Africa accounts for 94% of world malaria deaths, of which 67% occurred in children under five [1]. One of the primary goals of malaria therapy is the prevention of death.
production by bone marrow [21]. In this study, DL/D/P produced remarkable reduction in *P. berghei*-induced anemia characterized by increased serum RBC, HB, and PCV with decreased WBC than individual doses of DL, D/P, and CQ. Studies have shown that alterations in lipids may occur in pathological changes associated with infectious diseases including malaria [22]. Malaria parasite have been associated with elevated serum TG and decreased HDL-cholesterol levels in humans [20,23]. In the current study, serum TG, CHOL, and LDL-C were elevated whereas HDL-C levels were decreased in *P. berghei*-infected mice. However, treatment with DL/D/P restored the serum levels of the aforementioned lipids most than individual doses of DL and D/P. This observation shows that treatment with DL/D/P produced the best schizonticidal activity than treatment with individual doses of DL, D/P, and CQ. This observation may be attributed to the formation of a synergistic front with D/P through complementary antimalarial action. D/P is one of the currently recommended artemisinin-based therapy that has reduced morbidity and mortality associated with malaria. The antimalarial mechanism of dihydroartemisinin involves two steps. The first step involves the cleavage of the endoperoxide bridge and the generation of free radicals by intra-parasitic iron. The second step is the formation of covalent bond between the parasite proteins and artemisinin-derived free radicals [24]. The exact antimalarial mechanism of action of piperaquine is unknown, but studies suggest similar mechanism as CQ due to close structural resemblance. CQ accumulates in the parasite food vacuole where it binds free hematin resulting in the accumulation of toxic free CQ-hematin complex and hemoglobin within the food vacuole. The CQ-hematin complex disrupts the vacuole membrane and interferes with enzymatic processes in the parasite [25].

5. CONCLUSION

The observation in this study shows that DL/D/P produced the best curative, suppressive, prophylactic, and anti-anemic activities than individual doses of DL and D/P. Also, DL/D/P increased MST and restored lipid profile of parasitized mice most than individual doses of DL and D/P. This shows that DL/D/P may be an effective antimalarial drug combination, but further evaluation in humans is imperative.

6. ACKNOWLEDGMENT

The authors appreciate all the laboratory staff of the animal house of the Department of Pharmacology, University of Port Harcourt.

7. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

8. FUNDING

There is no funding to report.

9. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

10. ETHICAL APPROVALS

The experimental procedures were approved by the Research Ethics Committee of the University of Port Harcourt, Port, Harcourt, Rivers State, Nigeria.
11. PUBLISHER’S NOTE

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How to cite this article:
Georgewill UO, Ebong NO, Adikwu E. Antiplasmodial activity of desloratadine-dihydroartemisinin-piperaquine on Plasmodium berghei infected mice. 2021;9(2):169-173. DOI: 10.7324/JABB.2021.9217