

Haematological effects of chloroquine and naphthoquinone on *Plasmodium berghei* infected male mice

Nkereuwem ETUKUDO, Olufadekemi KUNLE-ALABI, Opeyemi AKINDELE, Folasade BOLARINWA

Abstract— Increasing reports of the re-emergence of chloroquine- and naphthoquinone-susceptible malaria has rekindled interest in these drugs. Both drugs have previously been reported to cause haematological derangements similar to those usually associated with *Plasmodium* infection. It is thus not clear if the haematological changes observed during malaria are solely due to the parasite or to treatments as well. This study was therefore conducted to investigate the effects of chloroquine and naphthoquinone on haematological indices in parasitized and non-parasitized male Swiss mice. Mice were infected using Anka 1 N-strain of the malaria parasite, *Plasmodium berghei*. Infection was confirmed by examining thin blood smears prepared using tail blood under oil immersion microscope. Chloroquine (5, 10, 15 or 20 mg/kg) or naphthoquinone (0.1, 0.5, 1.0 or 2.0 mg/kg) was administered daily intraperitoneally using Tween 20 as vehicle for seven days. Mice were then sacrificed under chloroform anaesthesia and blood was collected through cardiac puncture. Parasitaemia was calculated by counting the number of parasites per total number of erythrocytes in a minimum of three random fields. Full blood count was carried out using standard procedures. Data were analysed using ANOVA and $p \leq 0.05$ was considered statistically significant. Erythrocyte, leukocyte and platelet counts; mean corpuscular volume; and mean corpuscular haemoglobin were significantly decreased in infected mice. Chloroquine and naphthoquinone produced more significant reductions of these variables in infected mice. However, parasitaemia was also significantly reduced in treated mice. The haematological effects of naphthoquinone and chloroquine are beneficial to combatting *Plasmodium* infection.

Index Terms— Chloroquine, Haematology, Mice, Naphthoquinone, Parasitaemia, *Plasmodium berghei*.

1 INTRODUCTION

Malaria, is a parasitic infection of circulating erythrocytes [1], [2]. Even though malaria-associated global morbidity and mortality have decreased substantially over the decades (due to increased distribution of insecticide-treated bed nets and increased availability of highly effective artemisinin combination treatments), malaria mortality has been estimated as about 2000 people per day since 2014 till present [3], [4], [5].

In humans, malaria is caused by five species of single-celled eukaryotic *Plasmodium* parasites (mainly *Plasmodium falciparum* and *Plasmodium vivax*) which are transmitted through the saliva of *Anopheles* mosquitoes [5]. One of the malaria-causing parasites, *Plasmodium berghei* was discovered in 1948 by Vincke in blood films of the stomach contents of *Anopheles durense* [6], [7], and is still very relevant in malaria transmission till date [8].

The major challenges with malaria eradication are the endemicity of the vector to certain regions (especially sub-Saharan Africa), and the propensity of the parasites to drug resistance [9], [10], [11]. Continual research to develop newer drugs to combat resistance led to the introduction of chloroquine and naphthoquinones. The chemical structures of chloroquine and naphthoquinones are similar and the spread of

chloroquine-resistant malaria discouraged their use for several years [12], [13], [14]. However, there have been reports on the return of chloroquine-susceptible malaria and of naphthoquinone use [12], [13]. The use of both drugs has been associated with haematological derangements [14]. It is however, not clear if the haematological changes observed during malaria are solely due to the parasite or to therapeutic interventions as well [15]. This study was therefore conducted to investigate the effects of chloroquine and naphthoquinone on haematological indices in parasitized and non-parasitized mice.

2 MATERIALS AND METHODS

2.1 Experimental Animals

Seventy male albino Swiss strain mice (18-22 g) obtained from the National Veterinary Research Institute, Vom, Plateau State, Nigeria were used. They were quarantined and acclimatized for four weeks before the commencement of the study and fed with pelletized mice grower and water *ad libitum* throughout the study period.

2.2 Experimental Design

Two similar studies; A and B, were conducted.

Study A

The animals were randomly allotted into nine groups of five

animals each as follows;

| | | |
|----------|---|--------------------------|
| Grp I | - | Control (Tween 20) |
| Grp II | - | 0.1 mg/kg naphthoquinone |
| Grp III | - | 0.5 mg/kg naphthoquinone |
| Grp IV | - | 1.0 mg/kg naphthoquinone |
| Grp V | - | 2.0 mg/kg naphthoquinone |
| Grp VI | - | 5.0 mg/kg chloroquine |
| Grp VII | - | 10.0 mg/kg chloroquine |
| Grp VIII | - | 15.0 mg/kg chloroquine |
| Grp IX | - | 20.0 mg/kg chloroquine |

Study B

The animals were randomly allotted into five groups of five animals each as follows;

| | | |
|-------|---|--|
| Grp A | - | Plasmodium berghei infected |
| Grp B | - | Plasmodium berghei infected + 5 mg/kg chloroquine |
| Grp C | - | Plasmodium berghei infected + 10 mg/kg chloroquine |
| Grp D | - | Plasmodium berghei infected + 15 mg/kg chloroquine |
| Grp E | - | Plasmodium berghei infected + 20 mg/kg chloroquine |

2.3 Drug Administration

Chloroquine and naphthoquinone were administered daily intraperitoneally using Tween 20 as vehicle for seven days.

2.4 Infection of mice with *Plasmodium berghei* parasite

Anka 1 N-strain of the malaria parasite, *P. berghei*, maintained in mice by serial passaging from mouse to mouse was used throughout the experiment. The *P. berghei* infected donor mice were obtained from the Nigerian Institute of Medical Research, Lagos, Nigeria. Each mouse was subsequently given standard intraperitoneal inoculums of *P. berghei* parasites using a 1 ml disposable syringe.

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- Nkereuwem Etukudo has a Ph.D. in reproductive physiology from the University of Ibadan, Nigeria. E-mail: uwemetuklab@yahoo.com
 - Olufadekemi Kunle-Alabi has a Ph.D. in reproductive physiology from the University of Ibadan, Nigeria. E-mail: mrskalabi@yahoo.com
 - Opeyemi Akindele has a Ph.D. in reproductive physiology from the University of Ibadan, Nigeria. E-mail: opeyemiakindele@gmail.com
 - Folasade Bolarinwa is a professor of physiology of the University of Ibadan, Nigeria. E-mail: adeyombo@yahoo.com

2.5 Determination of parasitemia

Tail blood was collected from infected mice and thin blood smears were prepared, fixed with methanol, and stained with a 1:10 dilution of Giemsa stain 1x phosphate buffer (pH 7.1). Parasites were visualized under a 100x oil immersion microscope.

2.6 Collection of blood samples

Mice were sacrificed under chloroform anaesthesia by placing each mouse in a desiccator with a piece of chloroform-soaked cotton wool placed inside. Blood samples were collected through cardiac puncture. Parasites were visualized under a 100x oil immersion microscope, and parasitemia was calculated by counting the number of parasites/total number of erythrocytes in a minimum of three random fields.

2.7 Statistical analysis

Data were expressed as mean \pm SEM and analysed using ANOVA. $p < 0.05$ was considered statistically significant.

3 RESULTS

3.1 Effect of naphthoquinone on some haematological indices in mice

There was a slight increase in the mean erythrocyte count in group IV compared with the control (I) and other test groups ie. II, III and V (Table 1). Leukocyte counts decreased in all the groups as compared to the control group; this decrease was most significant in group III (Table 1). Granulocyte counts were increased in all groups compared with the control group (Table 1). Haematocrit and haemoglobin concentrations were decreased in all groups compared with the control (Table 1).

The Mean Corpuscular Volume (MCV) was decreased in all test groups compared with the control group (Table 1). Lymphocyte counts and Mean Corpuscular Haemoglobin (MCH) were significantly decreased in all the test naphthoquinone (Table 1). Platelet counts were decreased in all groups except group II when compared with the control group (Table 1).

3.2 Effect of chloroquine on haematological indices in male mice

There was a dose-dependent decrease in erythrocyte and leukocyte counts with chloroquine administration (Table 2). Granulocyte and monocyte counts were also significantly increased in chloroquine-treated groups (Table 2). Chloroquine caused a dose-dependent reduction in haematocrit, haemoglobin concentration; lymphocyte and platelet counts (Table 2). Group VII showed significant increases in MCV and MCH (Table 2). Group VIII showed a significant increase MCHC compared to the control (Table 2).

3.3 Effect of chloroquine on haematological indices in *Plasmodium berghei* infected male mice

Red and white blood cell and platelet counts were significantly reduced by *Plasmodium berghei* infection (Table 3). Treatment of *Plasmodium berghei* infection with chloroquine further reduced these haematological indices (Table 3).

4 DISCUSSION

Plasmodium infection has severe detrimental effects on haematological status [3], [15]. Naphthoquinone and chloroquine are active therapeutic regimens against *Plasmodium* infection [3], [13]. In the process of performing their anti-parasitic roles, naphthoquinone and chloroquine may inadvertently affect normal body cells especially the red blood cells which are the target cells of the parasite. This study was therefore conducted to investigate the effects of naphthoquinone and chloroquine on haematological indices using *Plasmodium berghei* as the malaria model.

The reduction in erythrocyte count observed in the naphthoquinone group may be one of the mechanisms it employs in combatting the red blood cell infestation associated with *Plasmodium* infection. Naphthoquinone increases signal transduction in the erythrocyte [16], [17] and this action can modulate response of the erythrocyte to *Plasmodium* thus leading to removal of infected cells from the circulation.

The reduction in leukocyte count observed in the naphthoquinone treated groups may be an indication of improved immune response in these groups. *Plasmodium* infection is known to reduce leukocyte counts [18]. However, naphthoquinone inhibits the proliferation of aggressive cell types [19], and may thus act as an anti-inflammatory drug when used to treat *Plasmodium* infection. To further support this, granulocyte counts were increased, while lymphocyte counts were significantly reduced following naphthoquinone treatment. Cytokine modulation has been used experimentally to treat infective processes by regulating leukocyte (particularly phagocytic) function [20]. The effect of naphthoquinone on blood cell signal transduction may also be mediated via cytokine modulation.

Chloroquine showed similar actions to naphthoquinone, however, naphthoquinone, in addition to the foregoing, showed significant reductions in platelet counts. Such data should expectedly be worrisome; however, it is an added advantage. Naphthoquinone has been reported to prevent thrombosis through the inhibition of platelet aggregation as well as prevention of release of platelet-derived factors [21], [22]. *Plasmodium* infection has been associated with thrombosis [23], thus the antiplatelet activity of naphthoquinone may ameliorate this risk.

5 CONCLUSION

The adverse effects of naphthoquinone and chloroquine on haematological indices are apparently potent factors which are useful in combatting *Plasmodium* infection.

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Table 1: Effects of naphthoquinone on haematological indices in male mice

| Groups | I | II | III | IV | V |
|---------------------------|--------------|---------------|-------------|-------------|-------------|
| Erythrocyte | 5.38±1.73 | 5.06±0.28 | 3.98±0.66 | 5.54±0.84* | 5.12±0.62 |
| Leukocytes | 3.26±2.07 | 3.06±1.05* | 1.99±0.42** | 2.22±0.41* | 2.23±0.49* |
| Granulocytes (%) | 28.65±3.57 | 63.33±5.16* | 42.10±13.5* | 29.95±5.20* | 45.80±17.3* |
| Lymphocytes (%) | 62.18±5.34 | 25.53±1.86 | 46.50±14.0 | 54.38±3.02 | 40.70±14.6 |
| Monocytes (%) | 9.25±3.21 | 8.70±3.15 | 9.93±4.70 | 12.20±5.82 | 9.85±4.19 |
| Platelets | 264.50±88.20 | 282.75±120.00 | 81.75±24.5 | 154.00±22.6 | 176.00±99.5 |
| Haemoglobin concentration | 7.20±2.63 | 6.28±0.38* | 6.60±1.91* | 6.40±2.19* | 5.80±0.85* |
| Haematocrit | 26.15±8.77 | 23.08±2.00* | 20.50±2.88* | 21.48±2.54* | 22.78±3.36* |
| MCV | 49.00±5.23 | 45.50±1.91 | 47.50±5.26 | 37.75±2.22 | 44.50±3.70 |
| MCH | 13.33±1.50 | 12.25±0.79 | 14.18±1.82 | 9.73±0.65 | 11.48±0.93 |
| MCHC | 27.33±1.38 | 25.38±2.33 | 29.03±3.69 | 25.13±0.49 | 25.15±0.69 |

Values are expressed as mean ± SEM. *p < 0.05 compared with control (group I). **p < 0.01 compared with control (group I). n = 5.

Table 2: Effect of chloroquine on haematological indices in

male mice

Plasmodium berghei infected male mice

| Groups | Erythrocytes (million/mm ³) | Leukocytes | Granulocytes (%) | Lymphocytes | Monocytes (%) | Platelets | Haemoglobin (g/dl) | Haematocrit | MCV (fL) | MCH (Pg) | MCHC (g/dl) |
|--------|---|------------|------------------|--------------|---------------|---------------|--------------------|-------------|-------------|-------------|-------------|
| I | 5.71±1.17 | 3.90±1.72 | 28.00±3.77 | 59.08±5.51 | 10.75±1.04 | 370.00±145.90 | 7.75±2.14 | 28.43±7.32 | 48.65±4.81 | 12.45±0.53 | 27.10±1.21 |
| VI | 6.70±0.24 | 6.51±3.99* | 52.75±6.91* | 34.68±7.24* | 13.50±0.75 | 300.7±136.43* | 8.28±1.84 | 30.90±5.36 | 46.03±7.87 | 11.98±2.52* | 26.58±0.95 |
| VII | 5.50±0.27 | 3.83±1.09 | 32.78±4.90* | 49.85±3.42* | 15.98±2.05* | 352.75±88.82 | 7.40±0.49 | 28.70±1.92 | 52.25±2.22* | 13.50±0.42* | 26.80±1.15 |
| VIII | 3.69±0.36* | 2.94±0.60* | 35.33±18.92* | 44.73±13.29* | 10.90±3.02 | 259.50±26.10* | 4.58±0.90 | 16.38±2.12 | 44.13±2.32* | 12.35±0.96 | 27.67±1.68* |
| IX | 1.76±0.26* | 1.66±0.88* | 45.85±7.13* | 37.03±3.82* | 12.70±0.91 | 404.00±2.30 | 2.13±0.25 | 8.45±0.94 | 48.30±2.68 | 12.48±0.96 | 25.03±1.17* |

| Groups | Erythrocytes (million/mm ³) | Leukocytes | Granulocytes (%) | Lymphocytes | Monocytes (%) | Platelets | Haemoglobin (g/dl) | Haematocrit | MCV (fL) | MCH (Pg) | MCHC (g/dl) |
|--------|---|------------|------------------|-------------|----------------|-----------------|--------------------|-------------|------------|------------|-------------|
| I | 5.71±1.17 | 3.90±1.72 | 28.00±3.77 | 59.08±5.51 | 10.75±1.04 | 370.00±145.90 | 7.75±2.14 | 28.43±7.32 | 48.65±4.81 | 12.45±0.53 | 27.10±1.21 |
| A | 5.40±0.41* | 1.56±0.88* | 43.85±7.53* | 34.78±7.54* | 16.98±2.07* | 302.72±134.13** | 6.30±0.40* | 13.28±2.02* | 47.05±7.55 | 12.78±2.42 | 25.83±2.17 |
| B | 4.62±0.34*# | 1.68±0.88* | 44.95±7.23 | 35.68±6.24* | 15.88±2.02* | 304.71±116.13** | 6.50±0.29* | 9.85±0.65*# | 47.03±6.87 | 12.98±2.52 | 26.03±2.18 |
| C | 4.54±0.35*# | 1.69±0.72* | 44.85±7.15 | 32.68±7.26* | 16.78±2.05* | 308.73±146.23** | 6.40±0.39* | 9.45±0.44*# | 47.63±7.62 | 12.96±2.62 | 26.13±2.07 |
| D | 4.21±0.42*# | 1.46±0.81* | 45.75±7.13 | 34.48±7.44* | 15.99±2.25* | 308.57±126.13** | 7.10±0.43* | 8.25±0.74*# | 47.03±6.83 | 11.76±2.56 | 26.33±1.27 |
| E | 4.11±0.34*# | 1.76±0.58* | 44.85±7.33 | 16.98±1.05* | 320.74±116.33* | 7.50±0.43 | 7.45±0.91*# | 46.43±7.63 | 11.68±2.42 | 25.23±1.17 | |

Values are expressed as mean ± SEM. *p < 0.05 compared with control (group I). n = 5.

Values are expressed as mean ± SEM. *p < 0.05 compared with control (group I). n = 5.

Table 3: Effect of chloroquine on haematological indices in