EFFECTS OF CO-ADMINISTRATION OF ASCORBIC ACID ON SULPHADOXINE/PYRIMETHAMINE IN MICE INFECTED WITH PLASMODIUM BERGHEI

[1]Iniaghe L. O., [2]Emeka P. M., [3]Ogbunuga H.
[1] Department of Pharmacology and Toxicology, University of Benin
[2] Department of Pharmacology, King Faisal University, AL-AHSA, Saudi Arabia
[3] Department of Applied Biochemistry, Nnamdi Azikiwe University, Awka

Author for Correspondence
E-mail address: komeiniaghe@ yahoo.com , Tel +234 8022113816

ABSTRACT

Background: Co-administration of antioxidants in antimalarial therapy is a common practise in some parts of Nigeria. Antioxidants such as ascorbic acid and vitamin E are often included in malaria therapy; this is aimed at reducing the cytotoxic effects of free radicals generated during malaria infection or from anti-malarial agents. There is therefore the need to evaluate the benefits or risks of this practice. The effect of ascorbic acid on mice infected with *Plasmodium berghei* and treated with sulphadoxine/pyrimethamine was evaluated in this study.

Methods: The effects of sulphadoxine/pyrimethamine (500mg/75mg/kg body weight) alone, ascorbic acid (4mg/kg) alone, and in combination were evaluated in mice infected with *Plasmodium berghei*. Parasitaemia and haematocrit levels were monitored throughout the duration of the experiment.

Results:
Sulphadoxine/pyrimethamine caused a rapid clearance in parasitaemia (3,400 ± 45.88 parasites/µl of blood from second day post drug treatment to zero by the ninth). Reduction in parasitaemia with sulphadoxine/pyrimethamine and ascorbic acid combination was observed from the second day post drug treatment with complete clearance of parasitaemia occurring on the fifteenth day (28,893.45.19 ± 64.01 to 0 parasites/µl of blood). Administration of ascorbic acid alone did not reduce, but caused a steady increase in parasitaemia from the second day till the ninth day post drug administration) when 100% mortality was recorded (67,960.19 ± 86.71 to 150,640.96 ± 1,660.30 parasites/µl of blood). Normal saline neither caused decrease in parasite density nor protected the animals from mortality, which occurred by the eleventh day; parasitaemia increased from 50,058.93 ± 73.45 on day 2 to 156,328.12 ± 2,409.70 parasites/µl of blood by the ninth day.

Conclusion: Administration of ascorbic acid may not be beneficial in anti-malarial therapy with sulphadoxine/pyrimethamine.

Keywords:sulphadoxine/pyrimethamine, Plasmodium berghei, malaria, parasitaemia.

Introduction

Malaria is one of most widespread infectious diseases of our time, taking the lives of almost one million people a year, most of them in sub-Saharan Africa and under the age of five. It is the fifth leading cause of death worldwide and affects almost half the world's population (3.3 billion) [1].

Malaria is caused by five species of plasmodium parasites; *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale Plasmodium malariae and Plasmodium knowlesi* [2]. Other species of Plasmodium such as *Plasmodium berghei* and *Plasmodium lopharae* are used in experimental studies to induce parasitaemia in mice and birds respectively [3,4].

In humans, the Plasmodium parasite metabolizes haemoglobin resulting in the formation of reactive oxygen intermediates (ROI) [5]. Drugs which increase the levels of oxidative stress in the parasite may overwhelm ROI defense mechanisms and lead to parasite death. Several anti-malarial drugs are known to produce their effects via induction of oxidative stress in parasite [6].

Thus the oxidative stress imposed on erythrocytes by malaria parasites and, to some extent, by the antimalarial agents has led to the inclusion of antioxidants and nutritional supplementation in antimalarial therapy.

The high rate of replication of the malaria parasite and the consequent high demand for nucleotides as precursors for DNA synthesis makes it particularly sensitive to antifolates and folate synthesis inhibitors[6]. Sulphadoxine inhibits *de novo* synthesis of folate while Pyrimethamine is known to inhibit dihydrofolate reductase and the conversion of dihydrofolate to tetrahydrofolate. This synergism inhibits pyrimidines and thymidylate available for DNA synthesis in the malaria parasite.

Ascorbic acid is an antioxidant involved in scavenging free radicals and reactive oxygen species, in stabilizing the hydroxyl radical and regenerating tocopherol and glutathione to the active state. These functions work to halt peroxidation of cellular lipid membranes. However, it is also involved in reutilization pathways for pyrimidines and deoxyribose moiety and, therefore by virtue of its mechanism of action,

could have an antagonistic effect on sulphadoxine/pyrimethamine [7]. This study, therefore, evaluates the effects of co-administration of sulphadoxine/pyrimethamine and ascorbic acid in mice infected with *Plasmodium berghei*.

Objectives

The aim of this study was to determine the synergistic or antagonistic effect of co-administration of ascorbic acid and sulphadoxine-pyrimethamine in malaria therapy in mice using parasite clearance rate and PCV values as indices.

Materials and Methods

Materials

Sulphadoxine/pyrimethamine (Fansidar®), Roche, Switerland; ascorbic acid injection, La Beta manufacturers, China; 0.9w/v sodium chloride solution (normal saline), Unique Pharmaceutical Company, Lagos.

Methods

Animals/Parasites

Adult albino mice of both sexes (21-25g) were obtained from the Animal House of the Federal Vaccine Laboratory (Yaba, Lagos, Nigeria). The animals were maintained under standard environmental conditions and had free access to standard diet (Oladokun Feeds Plc, Ibadan, Nigeria) and water. Animals were acclimatized for two weeks and fasted overnight, before experiments. Approval for the use of animals in the experiments was obtained from the Ethical Committee of the College of Medicine, University of Lagos, Lagos, Nigeria.

Plasmodium berghei parasites were obtained from the Nigerian Institute of Medical Research (Yaba, Lagos, Nigeria). 0.1ml of blood was taken from the tail of the donor mice infected with *Plasmodium berghei* and diluted to 5ml with normal saline. Each experimental mouse was inoculated with 0.1ml infected blood solution, according to WHO standard, to give 10^7 parasite density.

Effect of sulphadoxine/pyrimethamine and ascorbic acid on parasitaemia and haematocrit

Animals were distributed into five groups of ten animals per group. Animals in group 1 which served as the normal control were neither inoculated with parasitized blood solution nor given any drug.

Groups 2, 3, 4 and 5 received 0.1ml of the infected blood and were treated with sulphadoxine/pyrimethamine (500/75mg/kg), combination of sulphadoxine/pyrimethamine (500/75mg/kg) plus ascorbic acid (4mg/kg), ascorbic acid alone and normal saline, respectively.

Parasitaemia and packed cell volumes (PCV) were monitored throughout the period of experiment.

Blood samples were collected either from the tail of the mice or via orbital puncture. Thick smears were prepared, stained with Giemsa stain and viewed under the microscope. Infected red blood cells were counted using the formula [8]

$$\text{Parasite/}\mu\text{l} = \frac{\text{WBC count /}\mu\text{l} \times \text{Parasite counted against 100 WBC}}{100}$$

Where WBC count/ µl was 8,000.

PCV was determined using the heparinized capillary tubes, microhaematocrit centrifuge and the microhaematocrit reader.

Data Analysis

Results obtained from this study were subjected to statistical analysis using the analysis of variance (ANOVA) to test for significance at 0.05 probability level.

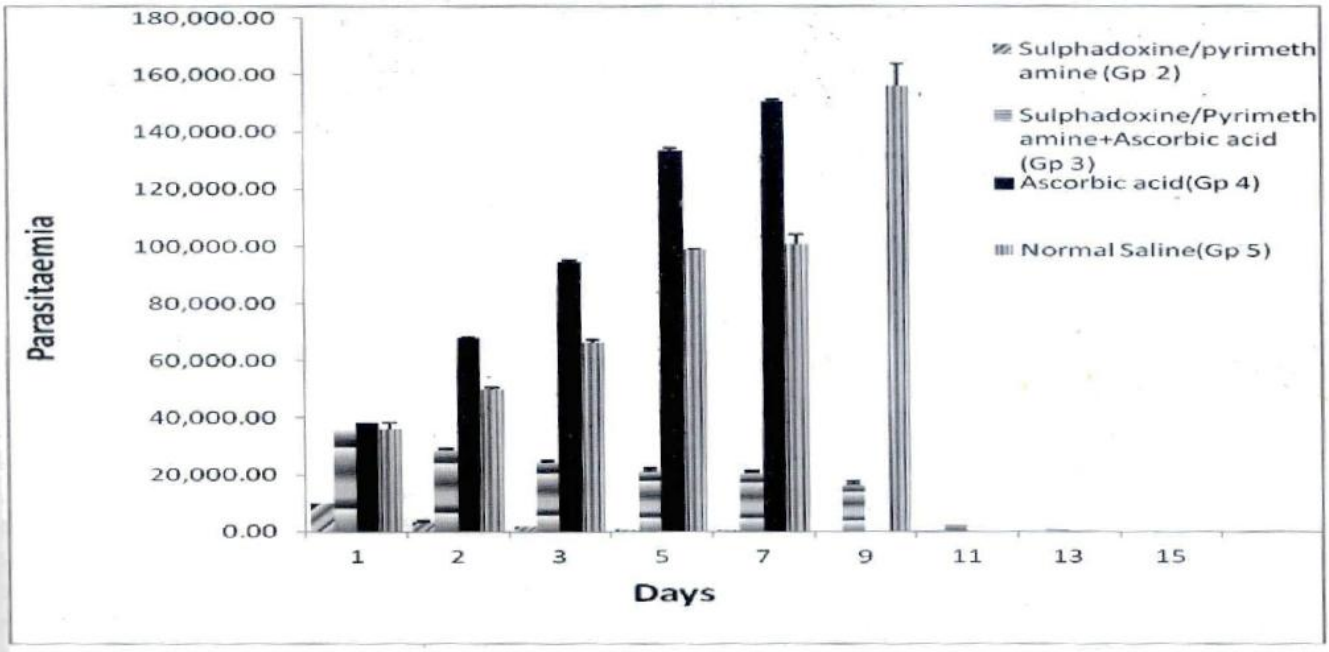

Fig 1: Mean parasite count for mice infected with *Plasmodium berghei* and treated with different drugs

Results

Figure 1 shows parasitaemia levels of mice infected with *P. berghei* and treated with the different drugs. Parasitaemia was not observed in the normal control.

In group 2, Parasitaemia levels declined from the second day post drug administration to the ninth day when zero parasitaemia was observed. 0% mortality was recorded in this group.

In group 3, reduction in parasitaemia was noticeable by the second day post drug therapy till the fifteenth day when no parasite was detected in blood samples. 30% mortality occurred in this group.

Parasite density in group 4 increased steadily from the first day post drug therapy ninth day when 100% mortality was recorded.

In group 5, parasitaemia also increased from day two to day eleven when 100% mortality was recorded.

The results of the statistical analysis showed that there was a significant ($p<0.05$) difference in parasitaemia between group 5 and group 2. There was however no significant ($p>0.05$) difference in parasitaemia levels between group 5 and group 3 and between groups 5 and 4.

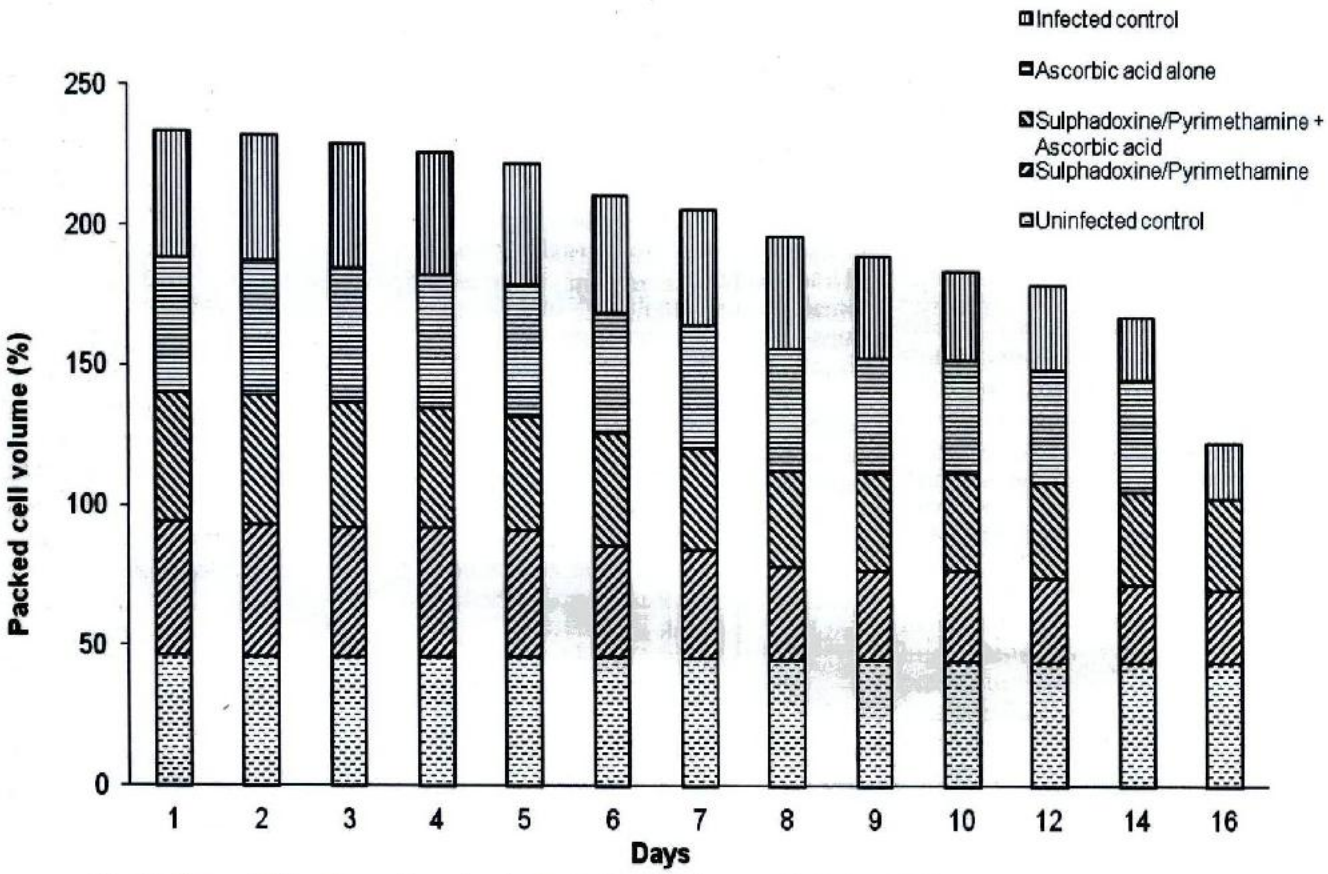

Fig. 2. Mean PCV values for mice infected with *Plasmodium berghei*

PCV values for test and control groups are shown in Figure 2. In group 1, a 5% reduction in PCV values was recorded (from 46.6 ± 0.64 on the first day to 44.0 ± 0.38 on the sixteenth day). In group 2, haematocrit levels steadily declined from 47.6 ± 0.72 on the first day and 25.8 ± 0.59 by the sixteenth day, indicating a 45% decline. In groups 3, 4 and 5 % reduction in PCV was 28.7%, 17.0%, and 56.0% respectively

There was a significant ($p<0.05$) difference in PCV values between groups 1 and 2. Significant ($p<0.05$) differences in PCV values were also observed between groups 1 and 3, and with groups 1 and 5. There was however no significant ($p>0.05$) difference in PCV values between groups 1 and 4.

Discussion

The results from this study show the complex effects of ascorbic acid in malarial therapy with Sulphadoxine/ pyrimethamine.

In animals treated with sulphadoxine/pyrimethamine, rapid clearance of parasites occurred. Reduction in parasitaemia levels was noticeable by the second day post drug administration with complete clearance of parasitaemia by the ninth day. Sulphadoxine/pyrimethamine is a synergistic drug combination often

employed to effect radical cure of blood schizonticide. It also acts as a strong sporonticide in the gut of mosquitoes when ingested with the blood of a host [9]. Though sulphadoxine/pyrimethamine is not a first line drug for treatment of malaria, it is recommended by WHO and used for intermittent preventive therapy (IPTp) in the second and third trimesters of pregnancy [10]. WHO also recommends that all infants at risk of *P. falciparum* infection in countries in sub-Saharan Africa with moderate to high malaria transmission should receive three doses of Sulphadoxine/pyrimethamine (IPTi) along with the Diphtheria (DTP2, DTP3) and measles immunization through the routine immunization programme [11]. In some countries where Artesunate-sulphadoxine-pyrimethamine remains particularly effective, WHO advocates this combination as a first line treatment [12]. Sulphadoxine/pyrimethamine was a useful alternative for Chloroquine resistant malaria and for patients who did not respond to Chloroquine [13], when the study was carried out. PCV levels of mice in this group were significantly ($p<0.01$) different from those treated with ascorbic acid, indicating the occurrence of haemolysis. Sulphadoxine/pyrimethamine has been associated with megaloblastic anaemia, hypersensitivity reactions and thrombocytopaenia [14]. The decline in PCV values could be attributable to inhibition of dihydrofolate reductase since sulphadoxine/pyrimethamine is known to reduce erythropoesis due to interference with folic acid metabolism [14].

Co-administration of sulphadoxine/pyrimethamine and ascorbic acid resulted in a decrease in parasite levels, though clearance of parasitaemia occurred at a slower rate than whensulphadoxine/pyrimethamine was administered alone. Reduction in parasitaemia was seen on the second day post therapy and complete cure occurred by the fifteenth day. This could be suggestive of soma antagonistic effect of ascorbic acid on the efficacy sulphadoxine/pyrimethamine in malaria therapy. Ascorbic acid is thought shield malaria parasites form oxidative stress induced by antimalarial agents. Ascorbic acid by virtue of its involvement in reutilization pathways for pyrimidines and deoxyribose sugars could antagonize sulphadoxine/pyrimethamine which inhibit pyrimidines and thymidylate available for DNA synthesis in the malaria parasite [7].

In ascorbic acid group, parasitaemia levels increased steadily form the second day following drug administration till the ninth day when 100% mortality was observed. This could be due to the protective effect of ascorbic acid on the malaria parasites to the detriment of the host. Antioxidants such as carotenoids, vitamin C and Vitamin E have been reported to protect malaria parasite from oxidative stress [15]. Ascorbic acid has also been shown to rejuvenate Vitamin E, making it an indirect contributor to fighting free radicals produced by oxidative stress [16, 17]. In another study, Vitamin E deficient mice were found to be resistant to *Plasmodium yoeli* infection while Vitamin E improved growth of Plasmodium in erythrocytes [18]. PCV values were not significantly reduced in mice treated with ascorbic acid (48.2 ± 2.01 on day one and $40.0\ 1 \pm 1.11$ by day sixteen) . This is consistent with the work of Onigbide and Iyawe who demonstrated that in parasitized conditions, ascorbic acid may induce erythropoesis in affected erythrocytes [19].

Conclusion

The use of ascorbic acid may not be suitable with concurrent administration of sulphadoxine/pyrimethamine in the management of malaria.

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