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IN VIVO EFFECT OF IRON SUPPLEMENT ON PARASITAEMIA IN MICE INFECTED WITH NK 65 STRAIN OF *PLASMODIUM BERGHEI* AND TREATED WITH ARTESUNATE: A PRELIMINARY STUDY

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Abstract

This study investigated the *in vivo* effect of iron supplement on parasitaemia in mice infected with NK 65 strain of *Plasmodium berghei* and treated with Artesunate. Several studies on the effects of mineral and vitamin supplements on artemisinin therapy in humans and mice models have reported conflicting results. Albino mice were infected with rodent parasite (NK 65 strain of *Plasmodium berghei*) and treated with either Artesunate (Art) only or with a combination of ferrous sulphate supplement (Fe) and parasitaemia was monitored for 3 days. Results indicated that parasitaemia was significantly ($p<0.05$) higher in the untreated group when compared to the treated and normal groups (no parasitaemia). In addition, challenged groups treated with artesunate only showed lower parasitaemia levels ($1.657\pm0.306 \times 10^3$) compared with those treated with artesunate and Fe ($5.368\pm1.484 \times 10^3$) or Fe only ($10.604\pm1.154 \times 10^3$). These findings suggest that Artesunate is an effective therapeutic agent in treatment of parasitaemia resulting from *P. berghei* infection and Iron supplementation reduces the therapeutic efficacy of artesunate in the treatment of parasitaemia resulting from P. berghei infection. The authors concluded that the use of iron rich multivitamin supplements should be with caution in the management of *P. berghei* infection using artesunate.

Keywords: *Artesunate, iron supplement, parasitaemia, mice, NK 65 strain Plasmodium berghei, malaria, red blood cells (RBCs)*

Introduction

Malaria is a mosquito-borne infectious disease affecting humans and other animals and is caused by haemoparasitic protozoans (World Health Organisation, 2016). There are five species of *Plasmodium* that may infect man, namely; *P. falciparum*, *P. vivax* , *P. ovale* , *P. malariae* and *P. Knowlesi* (Caraballo, 2014). Most deaths are caused by *P. falciparum* because *P. vivax* , *P. ovale* , and *P. malariae* generally cause a milder form of malaria (Lin *et al*., 2011) while *P. Knowlesi* rarely causes disease in humans (World Health Organisation, 2014). Malaria is a major endemic disease with high mortality rate in many tropical and subtropical countries especially in infants and young children. Malaria is one of the most prevalent, devastating parasitic infectious diseases in the third world countries. Forty percent of the world's population is at risk of infection in about 106 countries and in the year 2016 there was an estimated 216 million cases of malaria reported worldwide with an estimated 445,000 deaths worldwide (World Health Organisation, 2018).

Globally, the two regions with the highest prevalence and endemicity for malaria are Oceania and sub Saharan Africa. The greatest brunt of the disease is felt in sub-Saharan Africa where more than 90% of morbidity and deaths occurs in Nigeria and Democratic Republic of Congo and accounted for more than 35% of global malaria death in 2015 (World Health Organisation, 2018). Between 2000 and 2015, malaria incidence rate fell by 37% globally and by 42% in Africa. During this same period, malaria mortality rate fell by 60% globally and 66% in the African region (World Health Organisation, 2018). The burden of this disease is on the rise partly due to the increasing resistance of *Plasmodium falciparum* against the widely available antimalarial drugs. Chloroquine (CQ) was the mainstay of malaria treatment for many decades, but development of drug resistance by the parasite led to therapeutic failure (Thanh et al., 2012).

Artesunate is a membrane targeting semi-synthetic analogue of artemisinin capable of forming free radicals (Cui and Su, 2009; Lee et al., 2010). Artemisinins are generally more effective when used in combination therapies to combat plasmodium falciparum (Majori, 2004; Tall et al., 2007).

Plasmodium berghei is a unicellular protozoan that targets predominantly non-human hosts. We considered it a practical pathogenic laboratory species for understanding the humoral pathophysiology of *Plasmodium falciparum*, the causative agent of human malaria. In this study, the effects of Artesunate (an artemisinin derivative) on parasitaemia in mice infected with lethal strain *P. berghei* NK65, and treated with additional iron supplement albeit, on a short-term basis was evaluated.

Materials and Methods

Chemicals and solvents

Artesunate (98% artemisinin) and ferrous sulphate were purchased from Sigma-Aldrich, USA. Distilled water was extracted in our Laboratory.

Experimental Animals

ICR strain of mice of 6 - 9 weeks (25-35 g) were obtained from the breeding room of the Department of Biochemistry, College of Medical Sciences, University of Calabar, Nigeria. The animals were kept in standard cages measuring 25 cm x 10 cm with filter top in the 'Infected Animal room' at room temperature of 22- 24 ˚C with relative humidity of 45-65 % under 12 hours of day light: 12 hours of night light cycle. They were acclimatized for 7 days and maintained on standard feed, and were allowed access to water *ad libitum*. Experimental animals were kept in accordance with the guidelines for animal care as contained in the animal ethics handbook of the Faculty of Basic Medical Sciences, University of Calabar, Nigeria.

25 mice were randomly distributed into 1- 5 labeled cages of 5 mice. Mice were infected with previously maintained lethal strain of *Plasmodium berghei* strain NK65 (Waki et al., 1982) by intraperitoneal innoculation with 3 x 10^7 parasitized rat erythrocytes. The mice were shared into groups as follows: Group 1 = challenged + Art only; Group 2 = challenged + Fe only; Group 3 = Challenged + Art + Fe; Group 4 = negative control (challenged and untreated); Group 5 = Normal control. Artesunate (Art) was given at a dose of 4 mg/kg and Ferrous sulphate (Fe) was given at 65 mg/kg. Both

treatments were administered orally. Drugs were administered lasted for 3 days. The doses were adjusted in mice based on average body weight. Confirmation of parasitaemia was done by thin film microscopy 72 h after passaging.

Data Analysis

Results were presented as means ± SEM. Analysis of Variance (ANOVA) was used to analyze and compare means of the groups using SPSS software version 21.0. Variance means were separated with Turkeys Post hoc test. Differences in mean between treatment groups at $p<0.05$ were considered significant.

Results

From Table 1 above, mice infected and untreated (Group 4) had the highest level of Parasitaemia (13.630±0.858 x 10^3) while the challenged group treated with artesunate only (Group 1) had the lowest levels of Parasitaemia (1.657±0.306 x 10^3). Mice in Group 2 (treated with Fe only) and group 3 (treated with artesunate and Fe) showed no significant difference in levels of parasitamia from those in Group 4 (plasmodium-treated only).

Discussion

Several studies on the effect multivitamins supplements such as iron supplements on artemisinin therapy in mice model are often conflicting, with researchers reporting both beneficial and deleterious effects(Meshnick *et al*., 1989). The murine model has been very important in malaria research as it appears to be the most cited animal model in malaria research with laboratory animals because of their high fecundity relatively inexpensive to raise, enabling researchers to study the function of particular genes through several generations of offspring during a reasonable period of time (Meshnick *et al*., 1989; Yang et al, 1994; Oreagba and Ashorobi 2007). Their physiology and genetics have been studied extensively and can be compared to human easily (Meshnick *et al*., 1989). ICR strain of mice, are general-purpose mice, used extensively in infectious and pharmacological malaria research (Malenga *et al*., 2005).

Results of this study showed that artesunate administered alone resulted in significant reduction in parasitaemia values (1.657 x 10^3) in treated mice; and cleared the *P. berghei* parasites 2 days after completion of drug administration. This agrees with previous reports of artesunate as an established antimalarial, with confirmed efficacy (Malenga *et al*., 2005). However, when artesunate was administered concurrently with Fe, the rates of parasite clearance reduce suggesting a compromising effect of Fe. This observed interaction (antagonism) between artesunate and Fe was further confirmed in this study with the elevated levels of the parasitaemia in Fe only-treated group. The observed interaction for the concurrent administration of artesunate and Fe in this study has been reported by earlier by Meshnick *et al*. (1989) who demonstrated that antioxidant vitamins (such as α-tocopherol and ascorbate) interfere with the antimalarial activity of artemisinin derivatives. Also a study by Oreagba and Ashorobi (2007), showed that *in vivo*, retinol impairs the antiplasmodial activity of dihydroartemisinin (an artemisinin derivative) against *P. yoelii*.

Within the malaria parasite, host hemoglobin are degraded by a series of protease enzymes to release peptides and amino acids required for development and to create space within its digestive vacuole. During this process, a build-up of hematin occurs which is potentially toxic to the parasite. To circumvent this toxicity, the plasmodium parasite has developed a mechanism whereby hematin undergoes bio mineralization to form insoluble non-toxic hemozoin (malaria pigment). One of the first studies completed by the Meshnick group (2002), suggested that the bio activation of 1, 2, 4 trioxanes is triggered by iron (II) to generate toxic activated oxygen. Artemisinin affinity for parasite-infected erythrocytes over normal erythrocytes was rationalized by the iron- dependent bio activation of the Endoperoxide Bridge.

Studies have shown that the artemisinin is activated probably by Fe(III)PPIX complex to become a potent alkylating agent which reacts with both Fe(III)PPIX and proteins (Asawamahasakda *et al*., 1994; Bhisutthibhan *et al*., 1998). Since the Fe treated groups showed no significant activity, Meshnick (2002), Yang et al (1993) and Yang et al (1994) concluded that proteins were more important alkylation targets than Fe (III) PPIX.

Our recent work and result show that this activation occurs in the red blood cells not as a

result of external Fe ions in the system, but as a result of inbuilt heme- Fe(III)PPIX formation already available in the system. Thus, the administration of ferrous sulphate alongside artesunate produced no positive or significant effect

Conclusion

This study has shown that iron supplements impaired the activity of artesunate in the clearance of *P. berghei* infected mice. In the meantime the use of Fe supplements and multivitamins with high levels of iron in the management of anaemia that may be induced from severe malaria should be done with caution considering the risk of relapse from un-cleared parasitaemia, on the one hand and post parasitaemic anaemia on the other. This interaction observed in mice however discourages the co-administration of the both Artesunate and Fe as widely purported by clinicians.

References

1. Asawamahasakda W, Ittarat I, Pu YM, Ziffer H, and Meshnick SR. Reaction of antimalarial endoperoxides with specific parasite proteins. Antimicrob. Agents Chemother. 1994; 38:1854–1858.
2. Bhisutthibhan J, Pan XQ, Hossler PA, Walker DJ, Yowell CA, Carlton J, Dame JB, and Meshnick SR. The *Plasmodium falciparum* translationally controlled tumor protein homolog and its reaction with the antimalarial drug artemisinin. J. Biol. Chem. 1998; 273:16192–16198.
3. Caraballo H. Emergency department management of mosquito-borne illness: Malaria, dengue, and west Nile virus. Journal of Emergency Medicine Practice. 2014;16 (5): 7-14.
4. Lin JT, Bethell D, Tyner SD, Lon C, Shah NK, Saunders DL, Sriwichai S, Khemawoot P, Kuntawunggin W, Smith BL, Noedl H, Schaecher K, Socheat D, Se Y, Meshnick SRand Fukuda MM. *Plasmodium falciparum* gametocyte carriage is associated with subsequent *Plasmodium vivax* relapse after treatment. Public Library of Science One, 2011; 6 (4):18716.
5. Malenga G, Ayo P, Sarah S, Walter K, Theonest M, Evelyne A, Karen IB and Christopher JMW. Antimalarial treatment with artemisinin combination therapy in Africa. BMJ. 2005; 331:706-707.
6. Meshnick SR. Artemisinin: mechanisms of action, resistance and toxicity. Int. J. Parasitol. 2002; 32:1655–1660.
7. Meshnick SR, Tsang TW, Lin FB, Pan HZ, Chang CN, Kuypers F, Chiu D and Lubin B. Activated oxygen mediates the antimalarial activity of qinghaosu. Prog. Clin. Biol. Res. 1989; 313: 95-104.
8. Oreagba AI and Ashorobi RB. Interactions between retinol and some established antimalarials in *Plasmodium nigeriensis* infection in mice. Int. J. Pharmacol. 2007; 3:270-274.
9. Thanh VN, Hai DA, Hien DD, Takashima M & Lachance MA. *Moniliella carnis* sp. nov. and *Moniliella dehoogii* sp. nov., two novel species of black yeasts isolated from meat processing environments. International Journal of Systematic Evolutionary Microbiology. 2012; 62 (6):3088–309
10. World Health Organisation. Antimicrobial resistance: *Global report on surveillance. Geneva,* WHO Retrieved 2014
11. World Health Organisation. *Prevention of Malaria Infections. A practical Guide.* 2016; 4th edition pp. 75-86.
12. World Health Organisation. World Malaria Day, "Ready to Beat Malaria". Geneva: World Health Organisation. 2018
13. Yang YZ, Little B, and Meshnick SR. Alkylation of proteins by artemisinin. Effects of heme, pH, and drug structure. Biochem. Pharmacol. 1994; 48:569–573.
14. Yang YZ, Asawamahasakda W., and Meshnick SR. Alkylation of human albumin by the antimalarial artemisinin. Biochem. Pharmacol. 1993; 46:336– 339.

Table 1: Effects of artesunate and combination of artesunate and Fe on *Plasmodium berghei* strain NK65- induced Parasitaemia in mice (*n* = 5).

Group	Treatment	Parasitemia x 10^3
1	Plasmodium +Artesunate	$1.657\pm0.306 \times 10^{3a}$
2	Plasmodium + Fe	$10.604\pm1.154 \times 10^{3ab}$
3	Plasmodium + Artesunate+ Fe	$5.368\pm1.484 \times 10^{3ab}$
4	Plasmodium only	$13.630\pm0.858 \times 10^{3ab}$
5	Normal control	-

(Different Superscript indicates significant difference from normal control at $p<0.05$)