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Evaluation of the Antiplasmodial Effect of Retinol on *Plasmodium berghei berghei* Infection in Mice

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The present study was conducted to evaluate the antiplasmodial activity of retinol on chloroquine-sensitive *Plasmodium berghei berghei* infection in mice. Albino mice weighing 18-25 g where treated with retinol (50-200 mg kg^{-1}) in a set of experiments to investigate chemotherapeutic and prophylactic effect against *Plasmodium berghei berghei* infection in mice. Treatment in one of the chemotherapeutic groups continued throughout the study period. Retinol demonstrated a mild dose dependent schizontocidal effect on early and established infection. This effect became stronger on chronic administration but it also produced toxic manifestations and eventual death in most of the animals. Retinol also demonstrated a prophylactic effect by significantly delaying the onset of infection. The repository activity of retinol was however lower than that of the standard drug (Pyrimethamine-1.2 mg kg/day). Retinol possesses antiplasmodial activity especially during chronic administration thus suggesting that it might have a role in malaria control. Further studies in the area of its structural activity relationships are needed to justify this assertion.

Key words: Retinol, antiplasmodial activity, *Plasmodium berghei berghei*

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INTRODUCTION

Nutrition plays a major role in maintaining health and malnutrition appears to generate vulnerability to a wide variety of diseases and general ill health (Semba and Bloem, 2001). There are mixed opinions regarding how undernutrition affects susceptibility to malarial illness and mortality. Research in experimental animals suggest that some nutritional deficiencies e.g., essential amino acids, riboflavin and alpha-tocopherol will suppress the growth of malarial parasite (Lavander, 1993) However the co-existence of a high incidence of malaria and malnutrition suggest that malnutrition offers little protection against malaria.

Vitamin A plays an essential role in a large number of physiological functions including vision, growth, reproduction, hematopoiesis and immunity (Sommer and West, 1996) Vitamin A, is often deficient in individuals living in malaria endemic areas, and several studies reveal an association between vitamin A and malaria (Filteau *et al*., 1993; Galan *et al*., 1990; Das *et al*., 1996) and that it could play a part in potentiating resistance to malaria (Serghides and Kain, 2002).

Results from limited animal studies suggest that vitamin A deficiency may aggravate malarial infection. Using a rat model, Stoltzfns *et al*. (1989) showed that vitamin A deficiency decreases the body's ability to recover from malaria but only when the deficiency and infection occur early in life and the infection is very severe. It was also reported that vitamin A supplemented rats could better resist and recover faster from, malaria infection than vitamin A deficient rats. (Krishnan *et al*., 1976).

The effect of vitamin A supplementation on the incidence and severity of malaria infection was investigated by Binka *et al.* (1995) in a randomized trial in northern Ghana. Supplementing children 6 months to 5 years old with 200,000 IU of vitamin A every 4 months for 1 year did not affect the incidence of fever, malaria parasitemia rates, parasite densities, or rates of probable malarial illness. In another randomized, placebo controlled trial of vitamin A supplementation among children aged 6-60 months in Papua, Guinea, supplementation resulted in a 30% reduction in *Plasmodium falciparum* clinical episodes and 36% reduction in parasite density (Shankar *et al*., 1999) .

Apart from its ability to induce protection against falciparum malaria, limited reports have suggested that vitamin A may have a role in the treatment of malaria.

Although one study did not show retinol to be effective against *Plasmodium falciparum in vitro* (Samba *et al*., 1992), others found intrinsic anti-plasmodial activity of retinol at concentrations that were close to those in normal human serum (1-3 μM) (Davis *et al*., 1998) and even at higher concentrations (3-10 μM) (Hamzah *et al*., 2003). A recent study had gone further to characterize these effects *in vitro* (Hamzah *et al*., 2004).

There has however been no documented reports of similar *in vivo* studies and extrapolation to human malaria must be made cautiously.

The present study therefore aims to evaluate, through *in vivo* studies, the antiplasmodial effect of retinol in animal models.

MATERIALS AND METHODS

This study was conducted between September and December 2005 at the Chemotherapy Research Laboratory, Department of Pharmacology, College of Medicine, University of Lagos, Lagos Nigeria.

Laboratory animals: One hundred and sixty swiss albino mice (18-25 g) of either sex obtained from the Laboratory Animals Center, College of Medicine, University of Lagos, Nigeria were used for the various studies. They were kept in a well-ventilated environment, housed in standard cages and acclamatized for a period of ten days. They were maintained on standard pellets and water *ad libitum*. The mice were divided into four groups (A-D). The first three groups (A-C) were each divided into 5 subgroups of 10 mice per subgroup with one subgroup receiving the standard drug and another serving as control. The last group (D) consisted of just one subgroup of 10 mice.

Parasite: The NK65 strain of *Plasmodium berghei berghei* used in this study was obtained from Dr. O. Ademowo's Research Laboratory, College of Medicine University of Ibadan, Ibadan, Nigeria and maintained in mice by weekly passage. Each mouse was innoculated on day 0 intraperitoneally with 0.2 mL of infected blood containing about 1×10^7 *P. berghei beghei* parasitized red blood cells obtained from a donor mouse having about 60% parasitaemia.

Thin blood films were made by collecting blood from the tail, this was stained with Geimsa stain and the percentage parasitaemia was determined by counting the number of parasitized red blood cells out of 1000 blood cells in 10 random microscopic fields .

Drug administration: Retinyl palmitate (Sigma, USA) was dissolved in a vehicle consisting of Acetone, Tween 20 (Sigma, USA) and water in the ratio 0.25:5:4.75 (v/v/v) as described by Collins *et al.* (1992). Chloroquine powder

(Emzor Pharmaceuticals Ltd., Nigeria) and Pyrimethamine (SKG Pharma Ltd) were used as the standard drugs. All drugs used in the present study were administered orally with the aid of a stainless metallic feeding cannular.

Procedure: For Group A, the method described by Knight and Peters (1980) was used to determine the chemosuppressive effect of retinol. For Group B, determination of chemotherapeutic effect of retinol on established infection was carried out according to the modified method similar to the one by Ryley and Peters (1970). For Group C, the repository activity of retinol was evaluated using the method described by Peters (1965). Groups A to C received acute doses of retinol (50, 100 and 200 mg kg^{-1}) while Group D received chronic retinol doses (100 mg kg^{-1} daily, throughout the study period). The average percentage suppression of parasitaemia was calculated in comparison to control as shown below:

$$\text{Av.\% suppression} = \frac{\text{Av. \% parasitaemia in control} - \text{Av.\% parasitaemi in treated}}{\text{Av. \% parasitaemia in control}} \times 100$$

Statistical analysis: Data obtained from these studies were expressed as mean±SEM. Statistical analysis was performed using one-way ANOVA followed by Turkey Kramer post test. p-values less than 0.05 were considered statistically significant.

RESULTS

Retinol produced a mild, dose dependent schizontocidal effect on early and established *P. berghei* infection compared with the standard drug (Table 1). Chronic administration of retinol proved to be quite toxic to the parasites leading to their eventual clearance. (Fig. 1). However toxic manifestations were also observed in the animals leading to their death a few days after parasite clearance.

Table 1: Blood schizonticoidal activity of different doses of Retinol during early infection (4 day test)

Drug	Dose (mg/kg/day)	Average parasitaemia	Average suppression (%)
Retinol	200	5.43±0.25*	52.78
	100	5.63±0.15*	51.04
	50	6.77±0.31*	41.13
**Retinol Vehicle(Control)	0.2 mL	11.50±0.84	-
Chloroquine (Std)	5	3.40±0.56	70.43
One-way ANOVA	F	2.74	
	p	<0.05	

Data expressed as mean±SEM for 10 animals per group. df = 4,45, *p<0.05 when compared to control. Early infection - determined 4 days post inoculation. ** Equal volumes (0.1 mL) of drug and control vehicle were administered

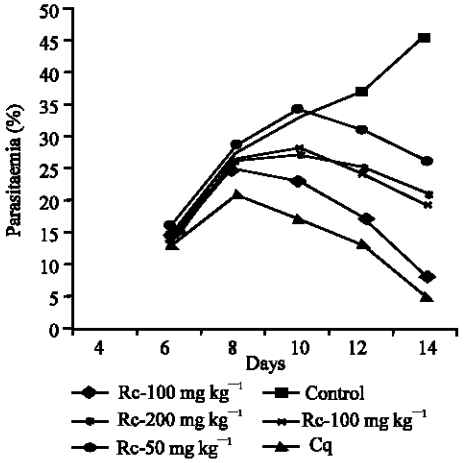

Fig 1: Effect of Different doses of Retinol on *P. berghei* infection in mice. Rc -100 mg kg^{-1} (Chronic Retinol Administration) means that 100 mg kg^{-1} was administered daily throughout the study period

Table 2: Prophylactic Effect of Retinol against *P. berghei* infection (Repository test)

Drug	Dose (mg/kg/day)	Average parasitaemia	Average suppression (%)
Retinol	200	3.45±0.35*	46.9
	100	3.84±0.35*	40.9
	50	4.86±0.19*	25.2
**Control (Retinol Vehicle)	0.2 mL	6.50±0.21	-
Pyrimethamine (Std)	1.2	1.40±0.03	78.5
One-way ANOVA	F	5.07	
	P	<0.05	

Data expressed as mean±SEM for 10 animals per group df = 4, 45 *p<0.05 when compared to control. Prophylactic Effect - determined 3 days post inoculation. **Equal volumes (0.1 mL) of drug and control vehicle were administered. One way ANOVA F = 5.07

Prophylactic administration of retinol caused a significant delay in the onset of infection compared with the control. It also caused a dose dependent repository activity at the various doses employed (Table 2). However the standard agent (Pyrimethamine) produced a higher chemosuppression compared to the retinol treated groups.

DISCUSSION

The present study was prompted by the observation in previous studies that retinol was directly toxic to *Plasmodium falciparum in vitro* (Davis *et al.*, 1998; Hamzah *et al.*, 2003) and that it possessed antiplasmodial activity against all stages of parasite development at high serum concentrations (Hamzah, 2004) Since acute

administration of large doses of retinol was toxic to our study animals (based on preliminary toxicity studies) we also investigated the effect of chronic administration of clinical doses of retinol on the parasite.

It was observed that retinol indeed possessed some degree of antiplasmodial activity as evidenced in the chemosuppressive effect on early and established infection. Death of the animals was probably due to the toxic effect of retinol on chronic administration (Wiegand, 1988).

The probable mechanism for the antiplasmodial effect of retinol could be through a direct cytotoxic action that involves binding of its metabolites to specific nuclear receptors known as retinoic acid receptors (RARs) and retinoid X receptors (RXRs) (Mange sdorf *et al*., 1990), this event could disrupt cellular differentiation and growth of the parasites. Evidence for this was shown in a recent study suggesting that the plasmodium parasite possesses a retinoic acid receptor-like moiety (Hamzah *et al*., 2003) An alternative mode of action could be through the production of oxidative stress to which the parasites are highly susceptible (Müller *et al*., 2003). Although retinol has powerful antioxidant effects (Schwartz *et al*., 1997) it can promote free radical generation in some systems (Murata and Kawanishi, 2000; Schwartz, 1996) and this could induce lipid peroxidation (Dal-Pizzol *et al*., 2001), an effect that proves quite destructive to the parasites. This effect is similar to the one that occurs with the endoperoxide moiety of artemisinin and its derivatives on *Plasmodium falciparum* culture. (Sibmooh *et al*., 2000). The protective effect of retinol observed in the present study has been demonstrated (*in vitro)* against *P. falciparum* in a prevoius study (Serghides and Kain, 2002) and the proposed mechanism was through the activation of the peroxisome proliferator-activated receptor (PPARg), a nuclear receptor that heterodimerises with RXR, leading to up-regulation of CD 36 expression and increased phagocytic capacity of macrophages for non-opsonised parasitised erythrocytes (Serghides and Kain, 2002). Non immune host which in this case include our study animals, rely on this innate phagocytic capacity as a first defence against malaria (McGilvray *et al*., 2000). Pre-incubation of red blood cells with high dose retinol in one *in vitro* study did not have any effect on merozoite invasion (Hamzah, 2004) indicating that its mechanism of protection was not due to red blood cell membrane effects.

Thus, large scale prophylactic administration of vitamin A to young children which is currently an integral part of National Immunization Programs in many developing countries may be beneficial for children living in malaria endemic areas. A controlled trial with vitamin A supplementation in Papua Guinea has confirmed this (Shankar *et al*., 1999).

The combination of Acetone, Tween 20 and water which was used as control vehicle for retinol greatly facilitated its absorption since aqueous dispersions and emulsions of vitamin A achieve higher plasma levels and at a faster rate than oily solutions (Bendich and Langseth, 1989). The control vehicle however seemed to also possess some antiplasmodial activity when compared to a group treated with distilled water alone, this may result in an exagerated antiplasmodial effect from possible synergism with retinol. Nonetheless these effects were mild as minimal volumes of drug was administered and therefore did not affect the overall findings.

The results of the present study has shown that retinol on chronic administration, displays antimalarial activity as seen in its ability to suppress *P. berghei* infection in mice. Its repository activity on the parasite has also been established *in vivo.* This confirms the findings from previous *in vitro* studies. Further studies are necessary to investigate its probable role in severe malaria. Elucidation of its structural activity relationship in this respect for more potent retinoids and its possible interactions with novel antimalarials (Skinner-Adams *et al*., 1999) in animal models will prove quite beneficial.

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REFERENCES

Bendich, A. and L. Langseth, 1989. Safety of vitamin A . Am. J. Clin Nutr., 49: 358.

Binka, F.N., D.A. Ross, S.S. Morris, B.R. Kirkwood, P. Arthur, N. Dollimore, J.O. Gyapong and P.G. Smith, 1995. Vitamin A supplementation and childhood malaria in northern Ghana. Am. J. Clin. Nutr., 61: 853-859.

Collins, M.D., C.I. Eckhoff, G. Bochert and H. Nau, 1992. 4-Methyl pyrazole partially eliminated the teratogeneicity of retinol and reduced the metabolic formation of all-trans retinoic acid in the mouse. Arch. Toxicol., 66: 652-659

Dal-Pizzol, F., F. Klamt, M.S. Benfato, E.A. Bernard and J.C. Moreira , 2001. Retinol supplementation induces oxidative stress and modulates antioxidant enzyme activities in rat sertoli cells. Free Radic. Res., 344: 395-404.

Das, B.S., D.I. Thurnham and D.B. Das, 1996. Plasma alpha-tocopherol, retinal and carotinoids in children with *falciparum malaria*. Am. J. Clinic. Nutr., 64: 94-100.

Davis, T.M.E., T.S. Skinner-Adams and J. Beilby, 1998. *In vitro* growth inhibition of *Plasmodium falciparum* by retinol at concentrations present in normal human serum. Acta Tropica, 69: 111-119.

Filteau, S.M., S.S. Morris and R.A. Abbott, 1993. Influence of morbidity on serum retinol of children in a community-based study in northern Ghana. Am. J. Clin. Nutr., 58: 192-197.

Galan, P., C. Samba, R. Luzeau and O. Amedee-Manesme, 1990. Vitamin A deficiency in pre-school age congolese children during malarial attack. Part 2: impact of parasitic disease on vitamin A status. Intl. J. Vitam. Nutr. Res., 60: 224-228.

Hamzah, J., T.S. Skinner-Adams and T.M.E. Davis, 2003. *In vitro* antimalarial activity of retinoids and the influence of selective retinoic acid receptor antagonists. Acta Tropica, 87: 345-353.

Hamzah, J., T.M.E. Davis, T.S. Skinner-Adams and J. Beilbyb, 2004. Characterization of the effect of retinol on *Plasmodium falciparum in vitro* Exp. Parasitol., 107: 136-144.

Knight, D.J. and W. Peters 1980. The antimalarial action of N-benzyloxy dihydrotriazines. The actions of Clociguanil against rodent malaria and studies on its mode of action Ann. Trop. Med. Parasitol., 74: 393-404.

Krishnan, S., A.D. Krishnan, A.S. Mustafa, G.P. Talwar, and V. Ramalingaswami, 1976. Effect of vitamin A and undernutrition on the susceptibility of rodents to a malarial parasite *Plasmodium berghei*. J. Nutr., 106: 784-791.

Lavander, O.A., 1993. Malarial parasites and antioxidant Nutr. Parasitol., pp: 107.

Mangelsdorf, D.J., E.S. Ong, J.A. Dyck and R.M. Evans, 1990. Nuclear receptor that identifies a novel retinoic acid response pathway. Nature, 345: 224-229.

McGilvray, I.D., L. Serghides, A. Kapus, O.D. Rotstein and K.C. Kain, 2000. Nonopsonic monocyte/macrophage phagocytosis of Plasmodium falciparum-parasitized erythrocytes: A role for CD36 in malarial clearance. Blood, 96: 3231-3240.

Müller, S., E. Liebau, R.D. Walter and R.L. Krauth-Siegel, 2003. Thiol-based redox metabolism of protozoan parasites. Trends Parasitol., 19: 320-328.

Murata, M. and S. Kawanishi, 2000. Oxidative DNA damage by vitamin A and its derivative via superoxide generation. J. Biol. Chem., 275: 2003-2008.

Peters, W., 1965. Drug resistance in *Plasmodium berghei* Vinke and Lips 1948: I Cloroquine resistance. Exp. Parasitol., 17: 80-89 .

Ryley, J.F. and W.Peters, 1970. The antimalarial activity of some quinolone esters. Am. Trop. Med. Parasitol., 84: 209-222.

Samba, D.C., L.K. Basco, F. Bleiberg-Daniel, D. Lemmonier and J. Le Bras, 1992. Absence of effect of retinol on the *in vitro* development of *Plasmodium falciparum*. Intl. J. Vitamin and Nutr. Res., 62: 99-100.

Schwartz, J.L., 1996. The dual roles of nutrients as antioxidants and prooxidants: their effects on tumor cell growth. J. Nutr., 126: 1221S-1227S.

Schwarz, K.B., J.M. Cox, S. Sharma, L. Clement, J.Humphrey, C. Gleason, H. Abbey, S.S. Sehnert and T.H. Risby, 1997. Possible antioxidant effect of vitamin A supplementation in premature infants. J. Pediatr. Gastroenterol. Nutr., 25: 408-14.

Semba, R.D. and M.W. Bloem, 2001. Nutrition and Health in Developing Countries. Totowa. NJ: Humana Press.

Serghides, L. and K.C. Kain, 2002. Mechanism of protection induced by vitamin A in *Falciparum* malaria. Lancet, 359: 1404-1406.

Shankar, A.H., B. Genton, R.D. Semba, M.Baisor, J. Paino and S. Tarnja, 1999. Effect of vitamin A supplementation on morbidity due to *P. falciparum* in young children in Papua Guinea: A randomised trial. Lancet, 354: 203-9.

Sibmooh, N.B., I.P. Pipitaporn, J. Wilairutl, R. Dangdoungjai, R.U. Looareesuiwan and L.T. Antharaksri, 2000. Effect of artemisinin on lipid peroxidation and fluidity of the erythrocyte membrane in malaria. BioI. Pharamcol., 23: 1275-1280.

Skinner-Adams, T., H. Barret and T.M.E. Davies, 1999 . Heterogenous activity *in vitro* of vitamin A (retinol) in combination with novel and established antimalarial drugs. Trans. Roy. Soc. Trop. Med. Hyg., 93: 550-551.

Sommer, A. and K.P. West, 1996. Vitamin A Deficiency: Health, Survival and Vision. Oxford Univ. Press New York.

Stoltzfns, R.R., F. Julal, P.N. Haevy and M.C. Nesheim, 1989. Interactions between vitamin A deficiency and P berghei infection in the rat. J. Nutr., 119: 2030-2037.

Wiegand, U.W., S. Hartmann and H. Hummler, 1988. Safety of vitamin A: recent results. Intl. J. Vitamin Nutr. Res., 68: 411-416.