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 were 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⁻¹). 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*
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 (Sembra 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 coexistence 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, Stoltzfus 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. Supplemeting 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 reacclimatized 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 inoculated on day 0 intraperitoneally with 0.2 mL of infected blood containing about 1×10⁷ P. berghei berghei 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 Giemsa stain and the percentage parasitaemia was determined by counting the number of paralyzed 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 0.25:5.475(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 cannula.

**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 therapeutic 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:

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\text{Av. % parasitaemia in control} - \frac{\text{Av. % parasitaemia 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.

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 antimalarial 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 antimalodal 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 adorf 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 (Sibmoch et al., 2000). The protective effect of retinol observed in the present study has been demonstrated (in vitro) against P. falciparum in a previous study (Sergides and Kain, 2002) and the proposed mechanism was through the activation of the peroxisome proliferator-activated receptor (PPARs), 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 (Sergides 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 (McGilravy 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 antimalodial activity when compared to a group treated with distilled water alone, this may result in an exaggerated antimalodial 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


