

ANTIPLASMODIAL ACTIVITY OF BP16524 AND SU 2242 AGAINST *PLASMODIUM BERGHEI* IN EXPERIMENTAL ANIMAL MODEL.

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Summary

The effects of two potential drug candidates: BP16524 [a synthetic analogue of cryptolepine] and SU 2242 [an enriched methanolic extract from *Enantia chlorantha*] on *Plasmodium berghei* were evaluated in this study. Investigations were performed using suppressive (early infection) and curative (established infection) test procedures. BP16524 showed significant dose dependent suppressive activity but was not effective against established infection. Significant ($p < 0.05$) activity was observed in SU 2242 (both for early and established infections).

Key words: BP16524, SU 2242, *Plasmodium berghei*, early and established infections.

Introduction

Malaria, a major threat to health constitutes an obstacle to economic development for individuals, communities and nations (particularly African and Asian countries). Almost half the world's population is at risk from this disease, which causes 300-500 million clinical cases and over 1.5-2.7 million deaths each year (Cheeseborough, 1999; WHO, 2000).

Due to current resurgence of malaria endemicity, of which a contributing factor is growing widespread resistance to antimalarial drugs (Bradley, 1996; Ramachandran, 2002), several organizations have launched programmes with the objective of developing new drugs. In the Medicines for Malaria Venture (MMV), the focus is also on new drugs, with the aim of developing one new antimalarial every 5 years (Ridley, 2001). The International Centre for Ethnomedicine and Drug Development (InterCEDD) in collaboration with International Cooperative Biodiversity Group in America, Europe and Africa,

have on-going projects for the identification and evaluation of new drugs from plant origin for treatment and prevention of malaria.

Several plants used in traditional medicine have been validated by scientific evidence for their efficacy and safety; there is therefore an upsurge in the interest in herbal remedies in several parts of the world (Okunzua, 1973; Keay, 1996; Okunji, 1996).

This study was therefore undertaken to screen two potential drug candidates from plant sources: BP16524 and SU 2242. BP16524 is a synthetic analogue of cryptolepine. Cryptolepine is the major bioactive compound isolated from the plant *Cryptolepis sanguinolenta*, a shrub grown in the rain forest and deciduous belt forest of the west coast of West Africa. Its main medicinal use is for the treatment of fevers and malaria.

SU2242 is an enriched methanolic extract of the plant *Enantia chlorantha*. *Enantia chlorantha* is a non-wood forest tree of the Annonaceae family that grows predominantly in West Africa, extending from

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Southern Nigeria to Gabon, Zaire and Angola.

Materials and Methods

Compound source and preparation

Both compounds (BP16524 & SU2242) used in the study were obtained from Walter Reed Army Institute of Research (WRAIR), Washington DC, USA while chloroquine base obtained from Kaycee Laboratories, Aba, Nigeria was used as the standard reference. The test compound (BP16524, 100mg) was dissolved in 10ml solution (made of 9.8ml normal saline and 0.2ml Tween 80) to give a stock solution of 10mg/ml and stored in a refrigerator at 4°C. Also the compound (SU2242, 100mg) was dissolved in 5ml of normal saline to give a stock solution of 20mg/ml. Further concentrations of 10mg/ml and 1mg/ml of BP16524 and 5mg/ml and 2mg/ml of SU2242 were diluted from the stock solution and used for the experiment. Chloroquine base (50mg) was dissolved in 10ml of normal saline to give a stock solution of 5mg/ml.

Animals

Swiss albino mice (18-35g) of either sex, bred and maintained under suitable conditions in the Malaria Laboratory, University of Port Harcourt, Rivers State, Nigeria were used for the experiments. The animals were fed with standard diet (Growers, from Topfeeds, Benin City, Edo State, Nigeria) and had access to water *ad libitum*.

Experimental procedure (antiplasmodial activity)

The mice were pre-screened to exclude any possibility of harbouring rodent *plasmodium* species by collecting blood from the tip of the tail and thick and thin films made. Antiplasmodial activity was assessed by the suppressive and curative test procedures.

Preparation of inoculum

Donor mice infected with rodent malaria parasite (*Plasmodium berghei* obtained from National Institute for Pharmaceutical Research and Development, Abuja, Nigeria) were anesthetized with chloroform and blood was collected via cardiac puncture. The pooled blood was diluted with normal saline and serum from uninfected mice such that 0.2ml of blood contained approximately 1×10^6

infected erythrocytes.

Suppressive test (BP16524 and SU2242)

This procedure involved treatment with the test compound immediately after the mice had been infected with the inoculum (Peters, 1970). Sixty four albino mice of either sex were chosen and divided into eight groups of eight mice each. The animals were given 0.2ml of the inoculum, intraperitoneally. The compound (2.5, 5, 10, 20, 40mg/kg) was administered subcutaneously once daily for four consecutive days, starting from the first day (D0). A parallel test was run using 5mg/kg Chloroquine (Kaycee, Nigeria) as reference and normal saline (0.2ml) as control. Thick and thin films made from tail blood on the fourth day (D3) were fixed with methanol, stained with Field's stain (A and B) and examined microscopically for parasitaemia. Average percent parasitaemia was calculated in comparison to control as shown below:

$$\text{Average \% Suppression} = \frac{\text{Average \% } P_{\text{control}} - \text{Average \% } P_{\text{treated}}}{\text{Average \% } P_{\text{control}}} \times 100$$

Where: P_{control} = parasitaemia in control group
 P_{treated} = parasitaemia in treated group.

Curative test (BP16524 and SU2242)

The experimental procedure for the curative test was similar to that for the suppressive test. The difference was in the administration of the compound, which required treatment with the compound after 72 hours (D3) of infection with inoculum. Different doses of the compound (2.5, 5, 10, 20, 40mg/kg), 5mg/kg of Chloroquine and 0.2ml of normal saline were administered subcutaneously to the different groups from days 3 to 7, during which parasitaemia level was monitored daily and mean survival times(days) were recorded.

Statistical analysis

All data were expressed as mean \pm SEM. The statistical analysis were performed using Student's 't' test. Values at $P < 0.05$ were considered to be significant.

Results

Suppressive test

As shown in Table 1A, the test compound BP16524 produced a dose dependent suppressive

effect. At 40mg/kg/day dose 83.87% suppression was obtained as against 87.10% suppression induced by the standard reference (5mg/kg/day Chloroquine).

Curative test

The curative activity of the compound is shown in Table 1B. There was a daily reduction in the parasitaemia level of the Chloroquine group; this was confirmed by the mean survival time value of 22.13 days. Mean survival time for the test compound had values ranging between 6.38 and 6.75, even less than the control group.

Table 1A: Effect of test compound BP16524 and Chloroquine (CQ) on *P.berghei* in mice. (Suppressive test)

Treatment	Dose (mg/kg/day)	Average Parasitaemia*	% Suppression
BP16524	40	0.15 ± 0.37	83.87
BP16524	20	0.23 ± 0.32	75.27
BP16524	10	0.35 ± 0/56	62.37
CQ	5	0.12 ± 0.05	87.10
NS**	0.2ml	0.93 ± 1.10	-

*Values are mean ± SEM, n = 8; ** NS, Normal saline.

Table 1B: Effect of test compound BP16524 and Chloroquine (CQ) on *P.berghei* in mice. (Curative test)

Treatment	Dose (mg/kg/day)	Mean Survival Time (Days)
BP16524	40	6.75 ± 1.73
BP16524	20	6.63 ± 1.72
BP16524	10	6.38 ± 1.67
CQ	5	22.13 ± 2.30
NS*	0.2ml	7.63 ± 1.55

*NS, Normal saline

Suppressive Test

From Table 2A, the average percent suppressive activity of SU2242 is shown, the test compound not only reduced parasitaemia level but also produced a dose- dependent chemosuppressive effect between 5mg/kg/day and 20mg/kg/day. At $p < 0.05$, doses 5mg/kg/day to 20mg/kg/day of test compound showed significant difference between treated group and control.

Curative test

In the established infection, it was observed that there was decrease in parasitaemia between

doses 5mg/kg/day and 20mg/kg/day. There was daily reduction in parasitaemia level of the Chloroquine group. This was confirmed by the mean survival time (MST) values as shown in Table 2A (Chloroquine had MST value of 21.14 days, while that for the test compound was between 13.86 and 16.29 days). These values were found to be significant at $p < 0.05$.

Table 2B: Effect of test compound SU2242 and Chloroquine (CQ) on *P.berghei* in mice. (Suppressive test)

Treatment	Dose (mg/kg/day)	Average Parasitaemia*	% Suppression
SU2242	20	2.68 ± 2.74	81.47
SU2242	10	4.60 ± 4.20	68.19
SU2242	5	6.17 ± 8.98	57.33
CQ	5	1.60 ± 2.16	88.93
NS*	0.2ml	14.46 ± 12.2	-

n = 7; *NS, Normal saline.

Table 2B: Effect of test compound SU2242 and Chloroquine (CQ) on *P.berghei* in mice (Curative test)

Treatment	Dose (mg/kg/day)	Mean Survival Time (Days)
SU2242	20	16.29 ± 1.87
SU2242	10	16.00 ± 1.90
SU2242	5	13.86 ± 1.23
CQ	5	21.14 ± 1.93
NS*	0.2ml	3.71 ± 1.49

n = 7; *NS, Normal saline

Discussion

In this study, results from the investigation showing a suppressive effect of 87.10% by Chloroquine is indicative of the fact that the parasite used was sensitive to the drug, mean survival time (22.13 days) also confirmed the efficacy of Chloroquine as a standard reference thereby ruling out influence of drug resistance in the results obtained.

In Test 1 (which involved the screening of BP16524) the test compound failed to show any schizonticidal effect in established infection, this was confirmed by the low mean survival time values for the different doses. This confirmed report from previous studies that cryptolepine and related alkaloids isolated from *Cryptolepis sanguinolenta* were active *in vitro* but failed to suppress established infection *in vivo* (Sharaf, 1993; Cimanga et al, 1997).

In the early infection (suppressive test), the various doses of the test compound effectively suppressed malaria infection. Studies have shown that cryptolepine and several substituted analogues were highly active against *Plasmodium berghei* in mice during suppressive test (Wright et al, 1996, 2001). Test 2 was the screening of SU 2242. Result showed significant chemosuppression both in the early and established infection, thus revealing that the test compound is a potential drug candidate.

Further studies need to be carried out to formulate drugs which can undergo clinical testing for efficacy as antimalarial drugs.

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