Effect Of Ablaquine Drug On *P.Berghei In Vitro*(II).

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الخلاصة

تهدف الدراسة الحالية الى تحديد زراعة النمو المتواصل لطفيلي الملاريا *P.herghei* بأستخدام احد الانماط المحسنة والملائمة مختبريا" ,كذلك دراسة تأثير علاج *Ablaquine* على طفيلي الملاريا *P.herghei* وتأثيره على حيوية ونمو الطفيلي .

استخدمت في الدراسة الحالية الاوساط الزرعية المختبرية ذات المدى القصير (short – term) بأستخدام عدة تراكيز مختلفة من علاج *Ablaquine* وهي : (10 – 100 – 250 – 500 – 1000 mg/ ml) مع عينة السيطرة في صحيفة الزرع ذات 24 حقل .

كانت نتائج الدراسة تشير الى حدوث التثبيط وحسب تراكيز العلاج المستخدم ,فكانت اعلى نسبة تثبيط 74 % سجلت في تركيز 1000 mg/ml تليها نسبة التثبيط التالية (32% - 47% - 58% - 60%) في تراكيز العلاج (10 – 100 – 250 – 500 mg/ml) على التوالي .

Abstract

The aim of the present study is to establishment of continuous culture of *P.berghei* using best suitable condition and also the effect of Aablaquine drug on *in vitro* invasion of *P.berghei* and the intracellular growth of the parasite .

A short – term culture using different concentrations (10 , 100 , 250 , 500 , 1000 , µg/ml) of Ablaquine drug along with controls was maitained in 24 well culture trays at 37 ^{o}C for 21 hr .

The invasion inhibition was found to be directly proportional to the concentration of drug used . 74 % inhibition was recorded with 1000 µg/ml concentration of Ablaquine drug followed by 60 % , 58 % , 47 % , 32 % inhibition caused by 500 µg/ml , 250 µg/ml, 100 µg/ml and 10 µg/ml respectivelly .

Introduction

Malara is one of the few diseases for which it is quick and simple to make an accurate biological diagnosis , even in a low-technology setting . Despite this clinical diagnosis in practiced widely , even through it has been shown repeatedly to be unreliable [1 , 2] .

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It is clear that new targets for drugs and vaccines are essential and that a better understanding of the biology of the parasite is prerequisite, but this is hampered by the complexity of the parasite life cycle [3].

In the past decade , however , CQ-resistant *P.vivax* has been reported from sever countries [4 ,5 ,6] .In thailand it was shown that *P.vivax* acquired infection can still be successfully treated with chloroquine [7].

Atovaquone in *in vitro* studies against *P.falciparum* has also shown varying results from antagonism with quinolines and artemisinin analogues to synergism with tetracycline and proguanil [8].

No adverse effects of artemisinin have yet been reported even in pregnant women[9].New antimalaria drug are urgently needed .

The use of short courses of the new antimalaria drug artemether as monotherapy has been limited by secondary malarial episodes following parasite clearance . Therefore a new antimalaria drug (CGP 56697, has been developed which combines artemether with a longer acting antimalarial agents , benflumetol [10].

No correlation was found between the responses to DU-1102 and chloroquine [11] In comparative efficacy trial of chloroquine(CQ) and Sulfadoxine-pyrimethamine (SP) for the treatment of uncomplicated falciparum malaria in Kampal, Uganda , Significantly higher levels of clinical and parasitological resistance to CQ than to SP has been found [12][13] ,found that the extensively used antimycotic drug clotrimazol drug clotrimzole (CLT) effectively and rapidly inhibited parasite growth in five different strain of *P.falciparum* , *in vitro* , irrespective of their chloroquine sensitivity .

Address 2 fundamental issue in the sex ratios of the rodent malaria parasite , *P . chabaudi.* The mortality rates was significatly higher for female gametocytes , with an average half-life of 8 h for female gametocytes and 16 h for male gametocytes [14].

Evaluation of three parasite lactate dehydrogen-sebased(PLDH) based rapid diagnostic test (RDTS)for the diagnosis of *faliciparum* and *vivax* malaria .

Non of the PLDH-based RDTS evaluated was able to detect non-falciparum malaria with high sensitivity . particallarly at low parasitaemias[15].

The aim of the present study is to establishment of continuous culture of *P.berghei* using best suitable condition and also the effect of Aablaquine drug on *in vitro* invasion of *P.berghei* and the intracellular growth of the parasite .

Materials and Methods

NK – 65 strain of Plasmodium berghei was maintained in white Swiss mice , Mus musculus (Balb/C) . 1-10 p.berghei infected erythrocytes were injeeted i. p. into naive mice and parasitaemia was monitored daily by preparing thin Giemsa – stained blood smears. Reticulocytosis

Short term *in vitro* culture:

Two types of culture medium were used for the short term *in vivo* culture . Incubation of culture using two different groups of red cells obtained from PHC treated mice and normal mice , short term *in vitro* culture was maintained in 24 well culture trays . (Laxbro india) . 4% hacmatocrit was prepared and lml . culture was incubated in 8 types of wells as described by [16 , 17] .

Long term *in vitro* culture

After establishing a short term *in vitro* culture , attempts were made to run long term *in vitro* culture of *P .berghie* using condition in which maximum invasion was observed i.e CM {RPM1-1640} with sodium bicarbonate {5% (w/v)} , HEPES {0.6%(w/v)} , gentamycin (50 µg/ml) , pencillin (100 iu/ml) and streptomycin (100 µg/ml) with additional glucose (mg/ml) } , NMS (5% Normal mouse serum) and RBC_r (PHC induced reticulocytes containing blood) .

Culture dishes were incubated at 37˚C in candle Jar . After every 24 hr one culture dish was terminated . Differential count of parasite stage i.e. rings , trophozoites and schizonts were done after every 24 hr till 168 hr .

Transfer of infected cells from *in vitro* to *in vivo*

Observations pertaining to course of parasitaemia *in vivo* was made by injecting $1x10^5$ infected red blood cells taken from *in vitro* culture dishes intraperitoneally to two normal mice. Thereafter the percent infection was monitored daily for ten days by preparing thin blood smear . The smears were fixed and stained and calculated for percent infection.

A short – term culture using different concentration (final concentration in 1.0 ml culture) of *Aablaquine* drug alongwith controls was maintained in 24 well culture trays at 37 ^oC for 21 hr .

The different concentration used were 10 µg/ml , 100 µg/ml, 250 µg/ml, 500 µg/ml, and 1000 µg/ml .

A stock of 10,000 µg/ml was prepared from which further dilution were made .Each concentration was added in duplicate wells while the contrls were added in triplicate wells .

Control wells were provided only with complete medium and haematocrit .After 21 hr incubation smears were prepared from each well .Smears were then air dried , fixed in methanol , and stained with Giemsa's stain and their differential count was made .
Persent invasion inhibition was calculated as =

$$100 - \frac{\text{Number of rings in expermented}}{\text{Number of rings in control}} * 100$$

Observation

Observation of short term *in vitro* culture as described by [17] .

Transfer of infected cells from *in vitro* to *in vivo*

$1x10^5$ *P . berghei* infected red cells obtained from culture at 24 hr interval were injected in two normal mice daily . The appearance of parasite in these mice was monitored by studying Giemsa stained thin smears every alternate day . in all the cases parasite appeared on 2nd day .

Mice injected with 48 hr culture pellet showed a gradual increase in parasitaemia till day 6th , after which percent infection started declining . Mice injected with 72 hr pellet showed an increase in parasitaemia till day 4 only . After this the parasitaemia gradually decreased .

In all the other cases i.e. , from 96 hr to 168 hr , observations made on course of parasitaemia showed a gradual increase in percent infection till day 6 , after which it reduced slowly (Table 1fig. 1) .

Table -1: Course of parasitaemia (in vivo) after injection $1 * 10^3$ *P. berghei* infected erythrocytes taken at 24 hr interval from contiuous culture

Group	Course of parasitaemia (%)					
	Day 0	Day 2	Day 4	Day 6	Day 8	Day 10
48 hr	1.54	0.47	0.54	0.60	0.34	0.11
72 hr	1.48	0.19	1.12	0.64	0.56	0.53
96 hr	1.58	0.23	0.44	0.49	0.47	0.18
120 hr	1.28	0.26	0.58	0.58	0.53	0.50
144 hr	1.08	0.18	0.57	0.58	0.55	0.14
168 hr	0.87	0.50	0.30	0.24	0.26	0.18

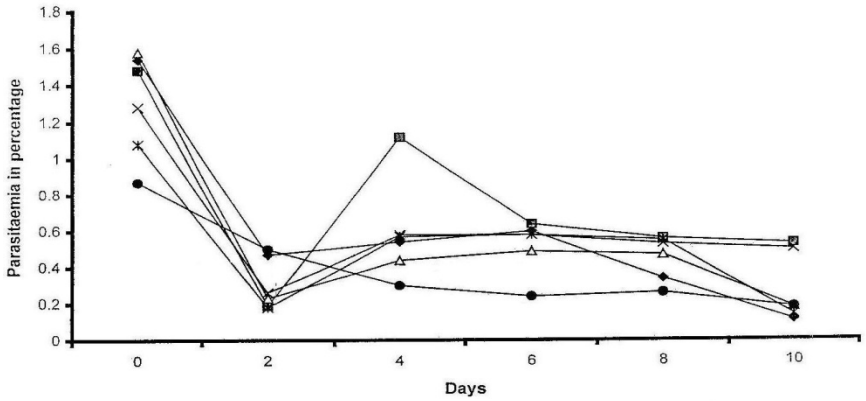

Figure -1: Course of parasitaemia (*in vivo*) after injecting 1 × 10^5 *P. berghei* infected erythrocytes taken at 24 hr interval from continuous culture

Observation regarding the effect of different concentrations of Aablaquine drug alongwith contrls on invasion of *P.berghei* into red blood cells were recorded . The concentrations used were 10 µg/ml , 100 µg/ml, 250 µg/ml, 500 µg/ml, and 1000 µg/ml (Fig.2) .

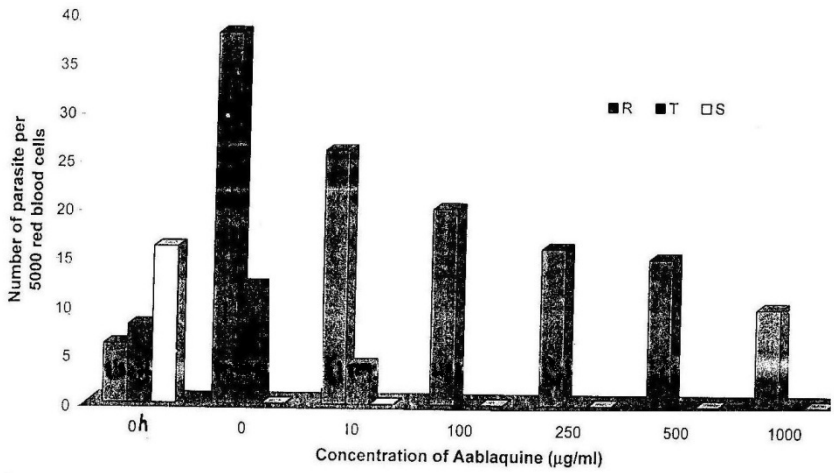

Figure. 2 Histogram showing effect of different concentrations of Aablaquine drug on the *in vitro* invasion of mouse erythocyte by *P.berghei*

The invasion inhibition was found to be directly proportional to the concentration of drug used . 74 % inhibition was recorded with 1000 µg/ml concentration of Aablaquine drug followed by 60 % , 58 % , 47 % and 32 % inhibition caused by 500 µg/ml , 250 µg/ml , 100 µg/ml and 10 µg/ml respectivelly .Moreover , there was reduction in the number of mature stages of parasite , troghozoite and schizonts after invasion as compared to 0 hr smears .

Discussion

Resistance to antimalarial drug is proving to be a challenging to problem in malaria control in most parts of the world . Resistance to chloroquine by now , has spread to most parts of the world . Chloroquine acts only on the erythrocytic stage of the plasmodium .However , there are dormant forms of parasites (hypnozoites) in the liver which can multiply and produce relapse . Primaquine , an 8 – aminoquinoline , has been the only drug available for the eradication of the dormant hepatic stages of *P.vivax* malaria . This drug is used as an anti-relapse agent in *P.vivax* malaria . This drug is used as an anti- relapse agent in *P.vivax* malaria . Though primaquine is highly effective as a radical cure , it is not without side effects . The most commonly cited side effect is methaemolobinaemia in patient with G6PDH(glucose – 6 – phosphate dehydrogenase) deficiency . (Aablaquine ™ product monograph) .

Recently , Aablaquine an 8 – aminoquinoline tissue schizontocide , a new generation antimalarial has shown promising effect against management of *P.infection* in the light of above facts , present study is carried out to check the effect of Aablaquine as blood schizontocide . *In vitro* invasion inhibition of 74 % was observed with 1000 µg/ml concentration of drug which decreased to 32 % with 10 µg/ml concentration . The effectivity of this drug as blood schizontocidal needs to be proped further .

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