

IN VITRO STUDIES OF ANTI MALARIAL DRUG RESISTANCE IN *PLASMODIUM FALCIPARUM*

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Abstract: The *in vitro* efficacy of four anti malarial drugs; chloroquine, basoquine, fansidar and fansimef was assessed against *Plasmodium falciparum* (blood stream forms) from the blood of 96 patients. The infected blood was diluted 10 times with RPMI 1640 medium and dispensed in each well of microtiter plate. Different concentrations of anti malarial drugs were made accordingly, added to the wells and incubated at optimum temperature in candle jar method. The parasites were counted under phase contrast microscope. *P. falciparum* from the blood of 24 patients were 62.5 % sensitive and 37.5 % resistant to chloroquine. Similarly they were 66.7 % sensitive and 33.3 % resistant to basoquine. While the parasites were 100 % sensitive to fansidar and fansimef from the blood of 24 patients for each drug.

Key words: *P. falciparum*, resistance status, *in vitro*, drug testing, anti malarial drugs.

INTRODUCTION

In vitro assays are quicker and reliable method to test drug resistance levels since it eliminates several host factors that interferes the results. *In vitro* testing is complementary to *in vivo* tests and their results are directly associated with the response of causative agents to drugs but both, *in vitro* and *in vivo* tests for resistance have their limitations and in any case do not measure the same biological phenomenon (Ringwald and Basco, 1999). The methods of detecting the presence of resistant strains of *Plasmodium falciparum* have been advanced by the use of *in vitro* techniques developed by Bruce-Chwatt (1981).

The first report of drug resistance in *P. falciparum* to quinine, according to the modern criteria dates back in 1910 (Wernsdrofer and Payen, 1991). A comprehensive study from March 1990 to 1992 was carried out in Central African Island to evaluate the response of *P. falciparum* to different anti malarial drugs, which showed 16 % resistance to chloroquine, 14 % to sulfadoxine/pyrimethamine, 9 % to quinine and 6.5 % to basoquine. Whereas the main land areas the resistance was 9 % to chloroquine, 3 % to sulfadoxine/pyrimethamine and 2 % to basoquine (Benito & Roche, 1995).

In Pakistan, falciparum malaria resistant to chloroquine was first reported in 1981 in Sheikhpura, Punjab (Yousaf and Nadeem, 1996). The *in vitro* efficacy of dihydroartemisinin, chloroquine, quinine, mefloquine, halfantrine and pyrimethamine were assessed against *P. falciparum* in Cameroon. A significant positive correlation was observed between the responses to dihydroartemisinin and mefloquine and halfantrine, suggesting *in vitro* cross resistance. Artemisinin derivatives are highly effective against *P. falciparum* isolates that are resistant to other anti malarial drugs (Ringwald and Bickii, 1999). *In vitro* studies help us to screen existing and new anti

malarial drugs efficiently and in shortest possible time. The present work was aimed to evaluate the resistance level of *P. falciparum* against routinely used anti malarial drugs.

MATERIALS AND METHODS

A study was carried out in Kot Abdulmalik, Khairanwala, Manawala and Skikhi villages of Sheikhpura, highly endemic for falciparum malaria. A lots of cases of falciparum malaria were detected and cases with a single infection of *P. falciparum* (asexual stages) at least 500 parasites/ml blood were selected. Persons who have received 4-aminoquindline within the last 14 days or pyrimethamine and sulphonamides within last 28 days were excluded from the test. According to Bruce-Chwatt (1981) micro technique was used for drug testing based on the candle jar system for the continuous *in vitro* culture of *P. falciparum*.

The microtiter plates (96 well) were pre-coated for chloroquine and basoquine at different concentrations. Well 'A' was control, wells B-H represent different concentration line bases on a geometrical progression of 2^0 , 2^1 , 2^2 , 2^4 , $2^{5.7}$, 2^8 , 2^{16} and 2^{32} pmol. Similarly for basoquine concentration line from well B-H based on a geometrical progression of $2^{0.5}$, 2^1 , 2^2 , 2^3 , 2^4 , $2^{5.7}$, 2^8 and 2^{16} pmol. For fansidar and fansimef, well A was control; well B-H represent concentration line as 0.1, 0.2, 0.4, 0.57, 0.8, 1 and 2 pmol.

The total inhibition of growth at 4 pmol of chloroquine or basoquine in the well indicates susceptibility to standard chloroquine or basoquine treatments. Growth at 5.7 pmol or more concentration in the well indicates resistance to chloroquine or basoquine. Growth at 4 pmol per well but inhibition at 7 pmol, may still be compatible with a satisfactory response to the drug tested.

RESULTS

The infected blood of 24 patients was *in vitro* tested with different concentrations of chloroquine where *P. falciparum* showed 62.5 % sensitivity and 37.5 % resistance (Fig 1). The parasites were able to survive or propagate at lower concentrations (2-3 pmol). Similarly the parasites showed 66.7 % sensitivity and 33.3 % resistance to basoquine (Fig 2). *P. falciparum* were 100 % sensitive to fansidar and fansimef and the parasites were cleared even at lower concentrations (0.2 – 0.4 pmol) during the incubation time.

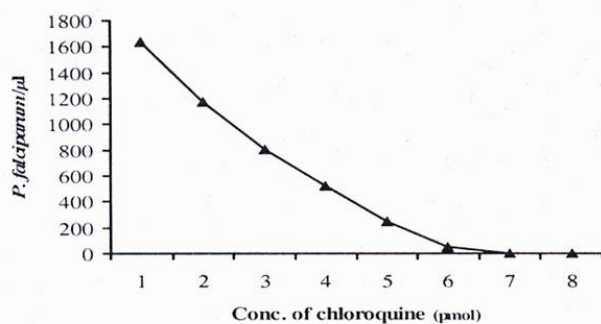


Figure 1. The response of resistant forms of *Plasmodium falciparum* against different concentrations of chloroquine.

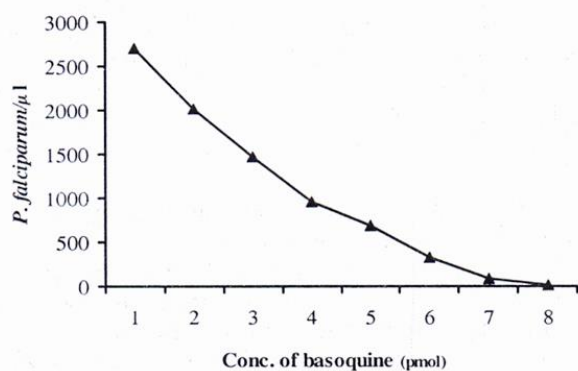


Figure 2: The response of resistant forms of *Plasmodium falciparum* against different concentrations of basoquine.

DISCUSSION

In the Eastern Asia the drug resistance was initially limited to relatively small areas of Indo-China Sub-continent. Later in 1970s it spread outwards and eastwards and by the year 1985 it reached up to the Arabian Peninsula. The first case of chloroquine resistance in *P. falciparum* in Pakistan was reported by the Directorate of Malaria Control, Islamabad in 1981.

It was documented by Verdrager (1986) that the resistance developed in *P. falciparum* was due to the presence of many factors in the habitat of falciparum malaria vector. Moreover, high drug pressure during intense transmission season

with mostly sub-curative doses of drugs and introduction of non immune populations in the areas. Some evidences as well indicate that the study area was periodically visited by people from India at Nankana Sahib near Sheikhpura. It is also evident that frequent movement of labour in the stone crushing industry located in the area contributed in the resistant strain of *P. falciparum* (Shah *et al.*, 1982).

During *in vitro* assays, lower doses are effective in clearing parasites. Since in *in vitro* testing only, the parasites are exposed to killing effects of the drug, they are left with no chance of producing surface membrane variants that can cope with the lethal effects of the drug. These variants are likely to be produced *in vivo* where not only the parasites have different hepatocellular cyclic stages but also the host reaction against the drug play a vital role in lowering the activity of the drug by detoxification and excretion processes. Moreover the absorption of the drugs at intestinal level (during oral administration) also varies from person to person, eventually alter the drug configuration or level in the blood. That may result in the variable response in different patients during *in vivo* testing. In *in vitro* testing such responses are excluded and the parasites are killed by the drugs. That is why the lower doses of anti malarial drugs are effective in clearing *P. falciparum* or inhibiting its growth *in vitro*. The lower doses are compatible to the dose given *in vivo*, so that the drug tested *in vitro* may be given in calculated doses to the patients. *In vitro* drug testing assays are recommended for quick and reliable screening of new drugs and evaluating the efficacies of the drugs in use.

In the view of increasing biological problems of drug resistance in malarial parasites, it is necessary to conduct a research with the objectives of elucidating the cause and to find the solution or alternative method of malaria control. So that resistance in *P. falciparum*, in the endemic areas of Punjab may not increase rapidly. The use of anti malarial drugs or any other curative measures may be implemented in such a way that the problem of drug resistance may not become a serious problem in future.

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