

In vitro Drug Sensitivity Testing of *Plasmodium falciparum*.

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BACKGROUND: In the previous Annual Report¹, the *in vitro* system for drug sensitivity testing of human *P. falciparum* utilizing radioactive isoleucine as a growth marker was described. The results indicated a low culture failure and a good reproducibility. Comparison of parasite C¹⁴-isoleucine incorporation and the morphology of the parasite during the course of maturity indicated an excellent correlation. The present investigation was done to obtain additional data on chloroquine sensitive *P. falciparum* for establishment of a base line growth curve for a susceptible strain.

It has been previously reported that strains of *P. falciparum* resistant to chloroquine are widely spread in Thailand. In some areas nearly 100% of the *P. falciparum* is resistant¹. The parasite source for cultures is primarily from Choburi Province, where the prevalence of resistant *P. falciparum* also approaches 100%². Results obtained from *in vitro* cultures support this observation, although the *in vivo* comparison has not been possible in every case.

PROGRESS: In the first part of this study a test for assessing the *in vitro* susceptibility of *P. falciparum* has been described. The culture system utilizes the protein incorporation of C¹⁴-isoleucine as a growth marker. Results obtained indicate a good reproducibility with the multiplication rate of 2:1 or better and culture failure was low. Patients admitted to Yala Provincial Hospital were used for *in vivo*-*in vitro* comparison studies, but the number of patients for a clinical follow-up was not sufficient to make an adequate number of observations on susceptible parasites.

Collaborative studies were established with the Faculty of Tropical Medicine, Mahidol University, Bangkok. Blood specimens obtained from patients with native *P. falciparum* were processed in *in vitro* culture utilizing the previously described technique. An aliquot of each specimen was washed and preserved in liquid nitrogen for later reference when required. A standard therapeutic course of chloroquine was administered to the patient and parasitemia was followed for evaluation of clinical response. The type of chloroquine resistance was established, utilizing WHO³ criteria for definition of resistance. Clinical response was later compared with the *in vitro* growth curve determined from C¹⁴-isoleucine uptake. The *in vitro* drug sensitivity test was performed on 35 blood specimens obtained from Somdej Sri Racha Hospital and the Faculty of Tropical Medicine, Bangkok. The growth curves obtained from the C¹⁴-isoleucine parasite incorporation were available from 25 *in vitro* cultures of which 20 were from Somdej Sri Racha Hospital and 5 from the Faculty of Tropical Medicine. Among these, 24 *in vitro* cultures were resistant to chloroquine and 1 was susceptible. Comparative results for *in vivo* and *in vitro* studies were obtained from 5 cases admitted to the Faculty of Tropical Medicine. The resistance of *P. falciparum* to chloroquine was observed both *in vitro* and *in vivo* in 4 cases. The patients were treated with other antimalarials after the assessment of chloroquine resistance. One case assessed as chloroquine susceptible *in vitro* is still under clinical

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observation. Parasitemia in this case lasted 24 hours after commencement of a standard therapeutic course of chloroquine.

It is obvious that the *in vitro* studies described have the limitation of depending on a natural human infection for the supply of infected blood. With the high prevalence of resistant infection, a base line growth curve of susceptible parasites will support the evaluation process of the *in vitro* test. Attempts are being made to obtain a sufficient number of samples of susceptible parasites to provide a reference for comparison with the quinine growth curve.

Additional studies are being done with the *in vitro* sensitivity test utilizing the currently reported effective antimalarials for comparison with quinine. The plasma levels for each antimalarial are being used as reference for the concentration to be tested *in vitro*. Antimalarials presently being tested are:

- 1) Pyrimethamine
- 2) Dapsone
- 3) Sulfadoxine
- 4) Sulfadoxine in combination with pyrimethamine
- 5) Primaquine phosphate

The morphological variations of the parasites observed during the incubation period in the presence of various antimalarials will be correlated with the growth curve of C^{14} -isoleucine incorporation. This observation, if possible, will be made on susceptible parasites and be correlated with the *in vivo* response.

REFERENCES:

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2. Personal communication.
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