Short communication

IN VITRO BROMODEOXYURIDINE INCORPORATION
ASSAY FOR DRUG SUSCEPTIBILITY OF
PLASMODIUM FALCIPARUM IN THE FIELD

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Received August 24 1989/Accepted October 22 1989

Drug susceptibility test using anti-bromodeoxyuridine (Doi *et al.*, 1988) was carried out with *P. falciparum* cases in coastal villages of North Sumatra, Indonesia between December 1988 and February 1989. The peripheral blood specimens were withdrawn from inhabitants with consent from vein and were put into culture in test tubes. The initial parasitemia were ranged 960-6,400/mm³. Blood samples containing parasites were transported in test tubes to Regional Health Laboratory of Medan. Experiments were carried out with 4 h culture and 16 h culture; 4 h culture was conducted in test tube at ambient temperature during transportation time, 16 h culture was done for 4 h in test tube at ambient temperature and for 12 h in candle jar at 37°C (Trager and Jensen, 1976). Experiments were conducted with 2 μ M of BrdU (Sigma, USA) in the presence of 0.5 μ M and 1.0 μ M of chloroquine concentrations (Aralen, Winthrop Products, USA). After BrdU flash, the ELISA was done according to our method described previously (Doi *et al.*, 1988). Chloroquine effect on DNA synthesis was monitored by measuring BrdU incorporation at 4 h and 16 h of incubation using ELISA.

Four hours culture of *P. falciparum* did not show any differences of BrdU incorporation under different concentration of chloroquine in 6 cases. The ring forms of 4 h culture could not be distinguished morphologically from pre-culture ones under microscopic observation. It may be mainly due to the shortage of time for the parasite to adjust to *in vitro* culture.

Results of 8 cases of *P. falciparum* demonstrated that drug susceptibility was distinguished by BrdU uptake when cultured for 16 h in this assay, which implied incorporation of BrdU into parasite nucleic acids (Fig. 1). At the chloroquine concentration of 0.5 μ M, BrdU uptake was inhibited in the range of 21-83%, and the mean of inhibition of BrdU uptake was 39%. *P. falciparum* free fresh blood was used as a negative control, and its ELISA value was 0.16 after BrdU flash. The value was the same as that of BrdU-free culture. Therefore, human leucocytes in whole blood did not incorporate BrdU in this assay.

The concentration of 0.2-0.3 μ M of chloroquine is the same level of patients blood administered that of 10 mg/kg. It is, thus, reasonable and feasible to monitor and judge chloroquine resistance at the concentration of 0.5 μ M. If only slight inhibition of BrdU uptake was observed at the concentration of 0.5 μ M chloroquine in isolated *P. falciparum*, chloroquine is not an effective drug against that strain *in vivo*.

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Figure 1 Inhibitory concentration of chloroquine for *P. falciparum* isolates from North Sumatra

In the present study, the drug effect on the proliferation of *P. falciparum* was monitored using BrdU incorporation in the field. This system does not need special equipments (Geary and Jensen, 1983; Waki *et al.*, 1989), and only portable spectrophotometer is enough. It would overcome the disadvantages of visually counting parasites (Desjardins *et al.*, 1979; Jensen *et al.*, 1981; Geary *et al.*, 1983). Small amount of blood (200 μl) from malaria patients is enough to test drug susceptibility in this assay. We confirmed the feasibility of our method in the field. It would offer a possibility of an accurate method of monitoring the effects of a variety of antimalarial drugs, and large-scale screening of drug resistance *P. falciparum* could be performed.

ACKNOWLEDGEMENTS

This investigation was supported by JICA (Japan International Cooperation Agency) and Ministry of Health, Republic of Indonesia. The authors are most grateful to Dr. Munthe, Dr. W. Panjaitan and Dr. T. Suzuki. Excellent technical assistance was given by the field workers especially, Mr. R. Siagian. We owe them all our best thanks.

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フィールドにおけるプロモデオキシウリジンの取り込みによる
熱帯熱マラリアの薬剤感受性試験

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我々は、先に発表したプロモデオキシウリジンの取り込みを測定する方法で、熱帯熱マラリアの薬剤感受性試験を、北スマトラ州東海岸のマラリア流行地にて行った。種々の薬剤濃度下で、普通の滅菌済み試験管を用い、患者の血液を16時間室温、室内で培養した。8例の患者から分離した熱帯熱マラリア原虫では、 0.5×10^{-6} Mのクロロキンの濃度で、プロモデオキシウリジンの取り込みが21-83%抑制された。この方法は、特殊な培養器具を必要とせず、分光光度計があれば実施できる簡便な薬剤感受性試験であり、マラリア流行地で広範な調査が可能であることを示した。

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