

NONISOTOPIC, SEMIAUTOMATED *PLASMODIUM FALCIPARUM* BIOASSAY FOR MEASUREMENT OF ANTIMALARIAL DRUG LEVELS IN SERUM OR PLASMA

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A simple, nonisotopic, semiautomated bioassay for the measurement of antimalarial drug levels in plasma or serum based on the quantitation of histidine-rich protein II in malaria culture is presented. The assay requires only small sample volumes and was found to be highly sensitive and reproducible. The results closely paralleled those obtained with isotopic bioassays ($R = 0.988$, $P < 0.001$) and high-performance liquid chromatography-electrochemical detection ($R = 0.978$, $P < 0.001$).

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PHARMACOKINETICS AND PHARMACODYNAMICS OF INTRAVENOUS METHYLENE BLUE IN HEALTHY RHESUS MONKEYS

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Methylene blue has antimalarial activity *in vitro* and *in vivo* in rats and humans. The purpose of this study was to characterize the pharmacokinetics and pharmacodynamics of intravenous methylene blue in healthy rhesus monkeys. Methylene blue 1% USP solution was administered as a single 30-second intravenous infusion at doses of 2.5, 5.0, 10 and 20.0 mg/kg to healthy rhesus monkeys ($n = 3/\text{dose}$). Serial venous blood samples were collected at 0, 5, 10, 20, 40, and 60 minutes, and 2, 3 and 5 hours postdose. Plasma and whole blood samples were analyzed for methylene blue concentration by HPLC-UV; plasma samples were also analyzed for total antimalarial activity by *Plasmodium falciparum*-based growth inhibition bioassay. All monkeys maintained normal clinical signs and symptoms except for blue feces and urine in the 10 mg/kg group for up to 4 days after dosing (pending results from 20 mg/kg dose). Methylene blue in healthy rhesus plasma demonstrates dose-dependent anti-malarial bioactivity when given as a single intravenous injection between doses of 2.5 and 10 mg/kg. Plasma antimalarial activities of methylene blue quantified by bioassay correlated well with their corresponding plasma concentrations quantified by HPLC ($r^2 = 0.97$, $n = 46$). However, methylene blue concentrated in the red cells with the ratio in whole blood to plasma of 50 at 20 minutes after dose administration. The ratio dropped rapidly to 12 at 1 h and to 2.0 at 5 h. Methylene blue concentration-dose profiles in plasma and whole blood demonstrated similar distribution profiles as well as similar pharmacological parameters of AUC and C_{max} . AUC increased directly with dose over the range of doses between 2.5 and 10.0 mg/kg ($r^2 > 0.71$, $p < 0.35$, $n = 6$). The rhesus whole blood dose-dependent relationship in this dose range is similar to humans with an interspecies scaling factor of approximately one. In conclusion, methylene blue was well tolerated in healthy non-infected rhesus monkeys, and demonstrated proportional increases in AUC relative to dose. The results of this study will be used to select two different doses of methylene blue to be used

in an antimalarial efficacy study of intravenous artesunate in combination with methylene blue in a *P. cynomolgil* rhesus monkey model of uncomplicated malaria.

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***PLASMODIUM FALCIPARUM*-BASED BIOASSAY FOR MEASUREMENT OF ARTEMISININ DERIVATIVES IN PLASMA OR SERUM**

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Artemisinin and its derivatives, artesunate and artemether, are rapidly acting antimalarials that are used for the treatment of severe and uncomplicated multidrug-resistant falciparum malaria. To optimize treatment regimens that use this new class of antimalarials, there is a need for readily available and reproducible assays to monitor drug levels closely in patients. A sensitive and reproducible bioassay for the measurement of the concentrations of artemisinin derivatives in plasma and serum is described. By modifying the in vitro drug susceptibility test, it was found that antimalarial activity in plasma or serum containing an unknown concentration of drug could be equated to the known concentrations of dihydroartemisinin (DHA) required to inhibit parasite growth. Dose-response curves for a *Plasmodium falciparum* clone (clone W2) and DHA were used as a standard for each assay. Assays with plasma or serum spiked with DHA proved to be reproducible (coefficient of variation, $\leq 10.9\%$), with a lower limit of quantitation equivalent to 2.5 ng of DHA per ml. For plasma spiked with artesunate or artemether, there was good agreement of the results obtained by the bioassay and the concentrations measured by high-performance liquid chromatography (HPLC) with electrochemical detection. The bioassay for measurement of the antimalarial activities of artemisinin derivatives in body fluids requires a smaller volume of plasma or serum and is more sensitive than the presently available HPLC methods, can provide pharmacodynamic parameters for determination of activity against the parasite, and should enhance the design of more appropriate dosage regimens for artemisinin drugs.

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