Quantitative Assessment of Antimalarial Activity In Vitro by a Semiautomated Microdilution Technique

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A rapid, semiautomated microdilution method was developed for measuring the activity of potential antimalarial drugs against cultured intraerythrocytic asexual forms of the human malaria parasite Plasmodium falciparum. Microtiter plates were used to prepare serial dilutions of the compounds to be tested. Parasites, obtained from continuous stock cultures, were subcultured in these plates for 42 h. Inhibition of uptake of a radiolabeled nucleic acid precursor by the parasites served as the indicator of antimalarial activity. Results of repeated measurements of activity with chloroquine, quinine, and the investigational new drug mefloquine demonstrated that the method is sensitive and precise. Several additional antimalarial drugs and compounds of interest were tested in vitro, and the results were consistent with available in vivo data. The use of P. falciparum isolates with known susceptibility to antimalarial drugs also permitted evaluation of the cross-resistance potential of each compound tested. The applications and expectations of this new test system within a drug development program are discussed.

The recent development of methods for the continuous in vitro culture of the human malaria parasite Plasmodium falciparum (4, 21) provides a valuable resource for the investigation of new approaches to malaria chemotherapy. Direct measurement of the antimalarial activity of potential new drugs against parasites grown in vitro is now feasible.

Resistance of P. falciparum to chloroquine is an important consideration in the treatment and prevention of malaria in many parts of the world, including South and Central America (12), Southeast Asia (25), India (20), and the African continent (6). It is therefore essential to use well-characterized susceptible and resistant strains of the parasite in the evaluation of potential new chemotherapeutic agents to detect cross-resistance with chloroquine and other standard antimalarial drugs.

The method described in this report can provide quantitative measurements of the antimalarial activity of large numbers of compounds, based on the inhibition of uptake of a radiolabeled nucleic acid precursor by the parasite during short-term cultures in microtiter plates. The parasites used are continually available from long-term maintenance cultures. The system is partially automated for rapid analysis of the data.

MATERIALS AND METHODS

Preparation of parasites. The parasite inocula used in these experiments consisted of two isolates of P. falciparum. The African Uganda I strain was originally obtained by Martin and Arnold from a child in Kampala, Uganda, in 1965 (9). It has been widely used in phase II clinical studies in human volunteers and has retained its susceptibility to chloroquine, quinine, and pyrimethamine. The Vietnam Smith strain, obtained in 1969 from an American soldier at the Walter Reed Army Medical Center, was first reported by Craig J. Canfield in 1971 (2). That patient, showing an R-I level of resistance to the combination of sulfaflane and trimethoprim after treatment failures with other antimalarial drugs, provided the parasite isolate designated Smith strain (2). This strain was later adapted to owl monkeys (24) and has also been used extensively in human volunteer studies, where its resistance to chloroquine, quinine, and pyrimethamine has been amply documented (10).

The two strains of parasites were grown continuously in stock cultures by a modification of the methods of Trager and Jensen (21) and Haynes et al. (4). A 6% suspension of human type A+ erythrocytes was prepared in culture medium which consisted of powdered RPMI 1640 (GIBCO Laboratories, Grand Island, N.Y.) diluted in sterile water with 25 mM HEPES (N-2-hydroxyethylpiperazine-N2-2-ethanesulfonic acid; Calbiochem, La Jolla, Calif.), 32 mM NaHCO3 (GIBCO), and 10% heat-inactivated (40 min at 56°C
and then clarified by centrifugation) human type A+ fresh frozen plasma (in acid-citrate-dextrose anticoagulant). Stock cultures were maintained in 5.0 ml of the 6% erythrocyte suspension in 25-ml tissue culture flasks (Corning Glass Works, Corning, N.Y.). The flasks were flushed with a gas mixture consisting of 5% O2, 5% CO2, and 90% N2 (Air Products Corp., Allentown, Pa.), sealed, and incubated at 37°C. Best results were obtained in individual experiments when the growth rate in the stock cultures was high as indicated by a doubling of the parasitemia every 24 h. This was accomplished by daily changes of the culture medium and by dilution with fresh erythrocytes every 2 or 3 days so that less than 2% of the cells were infected at any time. For each experiment, samples of the stock cultures were further diluted in culture medium containing sufficient noninfected type A+ human erythrocytes to yield a final hematocrit of 1.5% and parasitemia of 0.25 to 0.5% in preparation for addition to the microtitration plates.

Preparation of drugs. Preparation of compounds in a form suitable for addition to the microtitration plate at a known concentration required consideration of the aqueous solubility and sterility of each agent. Many compounds were relatively insoluble in water and had to be dissolved initially in an organic solvent. These problems were overcome by using a combination of sterile water and ethanol as the solvent. Each compound was first dissolved in a measured volume of either water or ethanol, which was then diluted with its counterpart to yield a 70% ethanol–30% water mixture containing a known concentration of the drug. This solution was allowed to stand at room temperature for 30 min. Further dilutions were then made by addition of the compound in the 70% ethanol solution to a measured volume of culture medium with constant mixing. Plasma proteins in the culture medium presumably provided binding sites for compounds which might otherwise have precipitated in an aqueous solution. The final dilution contained less than 0.1% ethanol, which had no measurable effect on the parasites in this system.

Preparation of microtitration plates. Microtitration techniques were used to measure the activity of a large number of compounds efficiently. The microtitration plate (Cooke Laboratory Products, Alexandria, Va.) used consisted of 96 flat-bottom wells, arranged in a matrix of eight rows (A through H) and 12 columns (1 through 12) (Fig. 1). The plates were prepared and the parasites were harvested by using strict aseptic techniques inside a laminar flow hood in the following sequence. An Eppendorf pipette or a Microdrop I (Cooke Laboratory Products) multiple-well filling pipette was used to place 25 μl of the culture medium in each well of the microtitration plate. Twenty-five microliters of the drug solution, prepared as described above, was then added to each of two adjacent wells in the second row (B) of the plate. Six compounds were thereby accommodated by each plate. After the drugs were added to the wells of row B, an Automatic Diluter (Cooke Laboratory Products) was used to make serial twofold dilutions across the plate in each column. When this was complete, row A remained free of any drug, and each of the drugs was present in duplicate columns at seven concentrations over a 64-fold range in rows B through H.

![Fig. 1. Representation of a microtiter plate, with 96 wells arranged in 8 rows (A–H) and 12 columns (1–12). When prepared as described in the text, wells 1 through 8 of row A serve as a parasite control (no drug present), and wells 9 through 12 of row A serve as an erythrocyte control (no drug and no parasites). Each compound is present in duplicate columns, over a 64-fold range, with the highest concentrations in row B and in twofold dilutions to the lowest concentrations in row H.](image)

A constant volume (200 μl) of the parasitized erythrocyte suspension described above was added to each well of the microtiter plate except the last four wells of row A, to each of which 200 μl of an equivalent suspension of nonparasitized type A+ human erythrocytes in culture medium was added. The total volume in every well was then 225 μl. The first eight wells of row A, containing no drugs, served as a parasite control. The last four wells of row A, containing neither drugs nor parasites, served as a nonparasitized erythrocyte control. The parasitized erythrocytes were exposed to each compound in two columns, with the highest concentration in row B and the lowest concentration in row H. Two plates were prepared for each experiment; the Uganda I strain of the parasite was added to one, and the Smith strain was added to the other.

After preparation as above, the plates were placed in a humidified air tight box (Instrumentation Department, Walter Reed Army Institute of Research, Washington, D.C.), which was then flushed with a gas mixture of 5% O2, 5% CO2, and 90% N2 and sealed. The box was then placed in an incubator at 37°C for 24 h.

Preparation of isotope and labeling of parasites. Uptake of [G-3H]hypoxanthine (Amersham/ Searle Corp., Arlington Heights, Ill.) was used as an index of growth of the parasites. The isotope is supplied as a lyophilate (1,000 mCi/mmol) in ampoules containing 1.0 mCi. The contents of a single ampoule were dissolved in 2.0 ml of 50% ethanol to provide a stock solution which was stored at −20°C. In preparation for addition to the microtitration plates, the ethanol was evaporated from a 0.2-ml sample of the stock solution, and 4.9 ml of culture medium was added to the remaining 0.1 ml of the isotope in water. The final isotope solution consisted of 20 μCi of [G-3H]hypoxanthine per ml of culture medium.

After the 24-h incubation period described above, the plates were removed from the box and 25 μl of the
isotope in culture medium (0.5 μCi) was added to each well. The plates were then returned to the box, which was again flushed with the 5% O₂-5% CO₂-90% N₂ gas mixture, sealed, and incubated at 37°C for an additional 18 h.

Harvesting parasites and scintillation counting. At the end of the second incubation period, each plate was harvested on a MASH II automated cell harvester (Microbiological Associates, Bethesda, Md.). This instrument aspirated and deposited the particulate contents of each of the wells onto small disks of filter paper (no. 934-AH, Whatman, Inc., Clifton, N.J.), which were then washed with copious volumes of distilled water. Each disk was dried and placed in a glass scintillation vial containing 10 ml of a toluene-based scintiﬂuor for counting. All 96 vials, corresponding to the 96 wells of the microtiter plate, were counted in a Searle model Delta 300 liquid scintillation spectrometer for a sufﬁcient period of time to ensure a counting error of less than 5% for each sample.

Data analysis. The counts and external standardization ratio for each vial were recorded on a paper punch tape for input to a desk top computer, the Tektronix Graphic System. The counts were converted by standard equations to disintegrations per minute for each well, which were tabulated in an 8-by-12 matrix corresponding to the 8 rows and 12 columns of the plate. The mean values for parasite control uptake and nonparasitized erythrocyte control uptake of [G-3H]hypoxanthine were calculated from the disintegrations per minute in row A, wells 1 through 8, and wells 9 through 12, respectively. The compound identifications and concentrations were entered at the keyboard of the computer. All concentrations were expressed as nanograms of the salt form per milliliter. The dihydrophosphate salt of chloroquine was used in every plate. The exact salt forms of most of the compounds were not identified at the time they were tested, so no attempt was made to calculate base weight or molar concentration. Unknown compounds were routinely diluted to provide an initial concentration of 125 ng/ml and reevaluated at higher or lower concentrations when necessary.

The automated system used in these experiments generated twofold serial dilutions with a 64-fold range of concentrations for each compound. The concentration-response curves for active compounds over this range were characteristically sigmoidal after logarithmic transformation of the concentration and were interpreted by nonlinear regression analysis. Concentration-response data for each compound were fit to a generalized sigmoidal function by the Marquardt algorithm (1), using a nonweighted least-squares criterion. The function used was a hyperbolic tangent function with four parameters: \( Y_i = (U - L)/2 \left[ 1 + \tanh (\beta \log C - \beta \log X_i) \right] + L \), where \( Y_i \) is the disintegrations per minute of the \( i^{th} \) sample, \( U \) is the upper asymptote of the function (approximated by the parasite control mean), \( L \) is the lower asymptote of the function (approximated by the nonparasitized erythrocyte control mean), \( \beta \) is a scaling parameter, and \( C \) is the concentration of agent corresponding to 50% inhibition of the uptake of [G-3H]hypoxanthine (the ID₅₀). This form of the logistic function was used because it provided convenient parameters for application to the in vitro test system (3). Estimates of each parameter were obtained by an iterative process which also provided a variance-co-variance matrix from which the 95% conﬁdence limits of the ID₅₀ were calculated. The computer output included a graph of the data with the fitted curve and accompanying estimate of the ID₅₀ (see Fig. 2-4). In addition, a variety of statistical tests were performed to determine the goodness of fit of each set of data to the model equation. These included calculations of the \( R \)-square and \( F \)-ratio for the regression and a nonparametric sign test on the residual errors.

RESULTS

Both the Uganda I and Smith strain parasites to chloroquine in one experiment, are shown in Fig. 2. An excellent fit of the data to the regression equation was obtained in each case. The ID₅₀ for chloroquine against the Uganda I strain of \( P. falciparum \) in this experiment was 10.3 ng/ml with a 95% confidence interval (CI) of 9.9 to 10.8 ng/ml. The resistance of the Smith strain to chloroquine was illustrated in this experiment by an ID₅₀ of 156 ng/ml (95% CI of 138 to 193 ng/ml). The asymmetry of the confidence intervals is related to the logarithmic transformation of the concentration in the model equation.

The effects of quinine on both strains of the parasite in this same experiment are illustrated in Fig. 3. As anticipated, considerable cross-resistance to chloroquine was exhibited by quinine, with an ID₅₀ in this experiment of 20.9 ng/ml (95% CI of 10.5 to 38.5 ng/ml) for the Uganda I strain compared with 135 ng/ml (95% CI of 111 to 162 ng/ml) for the Smith strain.

Mefloquine (WR 142,490), an investigational new antimalarial drug (22), was not cross-resistant with chloroquine or quinine in vitro (Fig. 4). The slope of response to mefloquine was similar to that seen with chloroquine. In this experiment, the ID₅₀ for mefloquine was 6.39 ng/ml.
FIG. 2. Comparative activity of chloroquine diphosphate against the African Uganda I strain and the Vietnam Smith strain of P. falciparum in vitro. The range of concentration of the drug was 0.95 to 125 ng/ml for the Uganda I strain and 19.5 to 1,250 ng/ml for the Smith strain. The disintegrations per minute represent the uptake of [G-3H]hypoxanthine by the parasites at each concentration of the drug. The ID_{50} was 10.3 ng/ml (95% CI = 9.9 to 10.8 ng/ml) for the Uganda I strain and 156 ng/ml (95% CI = 138 to 193 ng/ml) for the Smith strain. The graphs shown are part of the computer output described in the text.

(95% CI of 5.82 to 6.91 ng/ml) for the Uganda I strain and 6.66 ng/ml (95% CI of 6.43 to 7.19 ng/ml) for the Smith strain.

Multiple determinations of the ID_{50} for each of these three antimalarial drugs demonstrated the precision of the test system. The results of 12 separate experiments performed over a period of 3 months are shown in Table 1. As indicated, the mean ID_{50} for chloroquine was nearly 20 times higher with the Smith strain (182 ng/ml) than with the susceptible Uganda I strain (9.5 ng/ml). Considerable cross-resistance to quinine was indicated by the mean ID_{50} values of 109 ng/ml for the Smith strain and 26.1 ng/ml for the Uganda I strain. The lack of cross-resistance to mefloquine was also apparent, with a mean ID_{50} of 7.8 ng/ml for the Smith strain and 6.7 ng/ml for the Uganda I strain.

The three antimalarial drugs, amodiaquin, primaquine, and pyrimethamine, were evaluated in one experiment (Table 2). Amodiaquin, a 4-aminoquinoline similar to chloroquine, was
somewhat more effective than the latter drug against the Smith strain, with an ID$_{50}$ of 23.7 ng/ml. Primaquine, an 8-aminoquinoline used clinically for its effect on exoerythrocytic schizonts of Plasmodium vivax, was relatively ineffective in this test system, which specifically measures activity against only the asexual erythrocytic forms of P. falciparum. Pyrimethamine, a dihydrofolate reductase inhibitor with selective activity against the protozoan enzyme, was very effective against the Uganda I strain, with an ID$_{50}$ of 5.1 ng/ml. Against the Smith strain, which is known to be resistant to pyrimethamine in vivo, this drug was inactive at the highest concentration tested, 1,500 ng/ml.

Several of the many other compounds evaluated to date are presented in Table 3. Each of these compounds emerged from the U.S. Army Antimalarial Program and is a candidate for clinical studies. These results are generally consistent with existing in vivo data in animals and in some cases with clinical data in humans.
**DISCUSSION**

The adaptation of automated microtitration equipment described here provides a rapid and quantitative measurement of antimalarial activity for large numbers of compounds against *P. falciparum* cultivated in vitro. The potential value of in vitro drug susceptibility data for epidemiological purposes and in support of new drug development was discussed in a review by Trigg in 1976 (23). A microtitration system based on morphological assessment of the activity of chloroquine against parasites obtained from infected owl monkeys or humans was described and adapted for field use in epidemiological surveys by Rieckmann et al. (14). Although this method appears to provide reliable results with sufficient accuracy for certain applications, the more precise and rapid method using asexual parasites maintained in culture as described in this report offers many advantages for a new drug development program.

**Fig. 4.** Comparative activity of mefloquine hydrochloride (WR 142,490) against the African Uganda I strain and the Vietnam Smith strain of *P. falciparum* in vitro. The range of concentration of the drug was 1.95 to 125 ng/ml in both cases. The ID₅₀ was 6.39 ng/ml (95% CI = 5.82 to 6.91 ng/ml) for the Uganda I strain and 6.66 ng/ml (95% CI 6.43 to 7.19 ng/ml) for the Smith strain.
TABLE 1. In vitro antimalarial activity of chloroquine dihydrogen phosphate, quinine sulfate, and mefloquine hydrochloride (WR 142,490) against two strains of *P. falciparum*

<table>
<thead>
<tr>
<th>Drug</th>
<th>ID₅₀ (ng/ml)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>African (Uganda I)</td>
<td>Vietnam (Smith)</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>9.5 ± 0.78</td>
<td>182 ± 23.4</td>
</tr>
<tr>
<td></td>
<td>(7.8-11.2)</td>
<td>(130-233)</td>
</tr>
<tr>
<td>Quinine</td>
<td>26.1 ± 5.57</td>
<td>109 ± 8.5</td>
</tr>
<tr>
<td></td>
<td>(23.8-48.4)</td>
<td>(90.3-128)</td>
</tr>
<tr>
<td>Mefloquine (WR 142,490)</td>
<td>6.7 ± 1.00</td>
<td>7.8 ± 1.41</td>
</tr>
<tr>
<td></td>
<td>(4.5-8.9)</td>
<td>(4.7-10.9)</td>
</tr>
</tbody>
</table>

* All values are the mean ± standard error of 12 separate determinations.
* Value in parentheses are 95% confidence limits based on 12 separate determinations.

The use of a radiolabeled nucleic acid precursor as an indicator of parasite growth in an in vitro antimalarial drug screen was reported by McCormick and Canfield (11). By measuring suppression of the incorporation of [6-³¹C]orotic acid into deoxyribonucleic acid of *Plasmodium knowlesi* obtained from infected rhesus monkeys, the activity of several known antimalarial drugs was demonstrated. Suppression of the uptake and incorporation of [G-³¹H]hypoxanthine into nucleic acids by *Babesia* parasites in vitro has also been used as an indicator of drug activity (5). Though the feasibility of using the uptake of [G-³¹H]hypoxanthine to measure antimalarial activity in vitro has been demonstrated by the results in this report, the optimum conditions with respect to medium composition, level of parasitemia, duration of culture, and pulse time have not been fully evaluated. However, in a single experiment performed in this laboratory, there was good correlation between the ID₅₀ values for chloroquine and mefloquine as determined by morphological assessment and the isotopic method described here. Hypoxanthine, unlike thymidine, is capable of crossing the malaria parasite membrane (8). It is ultimately incorpo-

TABLE 2. In vitro antimalarial activity of amodiaquin, primaquine, and pyrimethamine against two strains of *P. falciparum*

<table>
<thead>
<tr>
<th>Drug</th>
<th>ID₅₀ (ng/ml)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>African (Uganda I)</td>
</tr>
<tr>
<td>Amodiaquin</td>
<td>9.8</td>
</tr>
<tr>
<td>Primaquine</td>
<td>543</td>
</tr>
<tr>
<td>Pyrimethamine</td>
<td>5.1</td>
</tr>
</tbody>
</table>

TABLE 3. In vitro antimalarial activity of several potential new antimalarial drugs against two strains of *P. falciparum*

<table>
<thead>
<tr>
<th>Compound*</th>
<th>ID₅₀ (ng/ml)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>African (Uganda I)</td>
<td>Vietnam (Smith)</td>
</tr>
<tr>
<td>WR 184,806</td>
<td>10.0</td>
<td>7.7</td>
</tr>
<tr>
<td>WR 190,409</td>
<td>35.4</td>
<td>48.5</td>
</tr>
<tr>
<td>WR 172,435</td>
<td>7.9</td>
<td>3.2</td>
</tr>
<tr>
<td>WR 171,669</td>
<td>2.5</td>
<td>3.9</td>
</tr>
<tr>
<td>WR 194,965</td>
<td>4.6</td>
<td>6.5</td>
</tr>
<tr>
<td>WR 99,210</td>
<td>1.4</td>
<td>1.8</td>
</tr>
<tr>
<td>WR 158,122</td>
<td>52.0</td>
<td>171</td>
</tr>
<tr>
<td>WR 225,448</td>
<td>&gt;1,500</td>
<td>&gt;1,500</td>
</tr>
</tbody>
</table>

* WR 184,806; DL-2,8-bis(trifluoromethyl)-4-[1-hydroxy-3-(N-t-butylamino)propyl]quinoline phosphate. WR 180,409: DL-three-α-(2-piperidyl)-2-trifluoromethyl-6-(4-trifluoromethylphenyl)-4-pyridine methanol phosphate. WR 172,435: 3-Di-n-butylaminol-1-[2,6-bis(4-trifluoromethylphenyl)-4-pyridyl] propanol methanesulfonate. WR 171,669: 1-[1,3-dichloro-6-trifluoromethyl-9-phenanthryl]-3-di(n-buty]l aminopropanol hydrochloride. WR 194,965: 4-(t-butyl)-2-[t-butylaminomethyl]-6-(4-chlorophenyl)phenol phosphate. WR 99,210: 4,6-diamino-1,2-dihydro-2,2-dimethyl-1-[3-(2',4',5'-trichlorophenyl)propoxy]-s-triazine hydrochloride. WR 158,122: 2,4-diamino-6-(2-naphthylsulfonyl)quinazolone. WR 225,448: proprietary compound.

The results presented in Table 1 clearly illustrate the ability of the test system to quantitate the extent of cross-resistance among antimalarial drugs. The excellent activity of mefloquine in vitro against a chloroquine-resistant strain of *P. falciparum* is consistent with its efficacy against infections with the same strain in owl monkeys (18) and with similar strains in clinical studies (22). Pharmacokinetic studies with this drug in humans show a peak blood concentration after a single 1,500-mg therapeutic dose of 1.2 to 2.0 µg/ml and a mean half-life of 14 days (R. E. Desjardins, C. L. Pampin, J. von Bredow, K. G. Barry, and C. J. Canfield, Clin. Pharmacol. Ther., in press). Based on these considerations and the information in the graphs of Fig. 4, the concentration of mefloquine in the blood is well above that required for complete inhibition of the parasite (0.1 µg/ml) for several days after a single oral dose of 1,500 mg.

Amodiaquin is thought to possess potentially useful activity against infections with chloroquine-resistant *P. falciparum*, based on clinical and experimental observations (19). The data in Table 2 support this impression and have led to interest in the development of new amodiaquin analogs. Also illustrated by the data in Table 2
is the known resistance of the Smith strain to pyrimethamine and the relatively poor blood schizonticidal activity of primaquine.

The compounds presented in Table 3 are of interest as potential new antimalarial drugs in various stages of investigation. One of these, WR 171,669, a 9-phenanthrenemethanol, has been shown to be effective against experimental infections with chloroquine-resistant \( P. falciparum \) malaria in human volunteers (15). The rest have been shown to be effective against chloroquine-resistant strains of \( P. berghei \) in mice (13) and against chloroquine-resistant strains of \( P. falciparum \) in owl monkeys. (17).

The compound WR 225,448 was quite active in vivo in owl monkeys against the Smith strain of \( P. falciparum \) (R. N. Rossan, unpublished data). It is therefore of interest that no activity could be detected in vitro at very high concentrations. The compound did not appear to precipitate either during preparation for testing or in the wells of the microtiter plate. It is characteristic of an in vitro test system that compounds which require in vivo metabolism to an active form will appear inactive in vitro. This possibility is being explored by in vitro metabolic studies with WR 225, 448.

Another compound of interest, WR 99,210, is a triazine derivative. The results with this compound (Table 3) are consistent with in vivo results in owl monkeys infected with pyrimethamine-resistant \( P. falciparum \) (16). The lack of cross-resistance between WR 99,210 and pyrimethamine is remarkable because of the presumed mechanism of action of both drugs as inhibitors of the enzyme dihydrofolate reductase. Another inhibitor of this enzyme, WR 158,122, a quinazoline, did show some cross-resistance with pyrimethamine in vitro (Table 3) and in vivo (16). The remaining compounds in Table 3, WR 184,806 (a quinolinemethanol), WR 180,409 and WR 172,435 (pyridine derivatives), and WR 194,965 (a phenylphenol) have all been evaluated for safety and tolerance in humans and are ready for phase II clinical efficacy studies.

When used as described, the present in vitro system is capable of detecting antimalarial activity for many compounds with various modes of action. However, it is likely that the absolute value of the ID\(_{50}\) for a given compound in vitro will vary somewhat among different laboratories because of differences in technique. It is therefore important when testing and reporting the results for a given compound to report also the results obtained simultaneously for known effective antimalarial drugs. Another important consideration with regard to the mechanism of action of a candidate antimalarial compound is the composition of the culture medium. The activity of sulfonamide drugs was not detected by the present system, presumably because of the abundance of \( p \)-aminobenzoic acid in standard RPMI 1640 culture medium.

In addition to its potential value both as a screen for new antimalarial drugs and for investigating the comparative activity of several analogs within a class of compounds, a number of other uses of the system are under consideration. The use of microtitration techniques in an 8-by-12 matrix of wells presents an excellent opportunity to evaluate potential synergy or antagonism among compounds. Combinations of compounds over a broad range of ratios of their respective concentrations can be generated by sequential dilution in two directions (across rows and columns). The ability of the system to assess the antimalarial activity in blood specimens obtained after administration of a drug to human volunteers is also being explored. It would be of considerable value, in conjunction with early clinical studies with a potential new antimalarial drug, to determine the kinetics of antimalarial activity in the blood of noninfected volunteers after drug administration.

The experimental approach to measuring the activity of potential antimalarial drugs against \( P. falciparum \) described in this report provides quantitative data not previously available and is sufficiently rapid and efficient to serve as a primary screen. It does not replace the very efficient primary mouse screen (7) or the secondary screen in owl monkeys (16). Rather, it provides supplementary information with respect to activity against the organism of interest, \( P. falciparum \), and allows more critical selection of the most active compounds within a given class. It has, in fact, become an integral part of the U.S. Army Antimalarial Drug Development Program.

**LITERATURE CITED**


