FIRST-TIME-IN-HUMANS SAFETY AND PHARMACOKINETICS OF WR 238605, A NEW ANTIMALARIAL

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Abstract. WR 238605 is an 8-aminooquinoline drug currently under development for prophylaxis and treatment of malaria. Preclinical studies have demonstrated that it has greater efficacy and less toxicity compared with primaquine. In this first-time-in-human randomized, double-blind, placebo-controlled study designed to evaluate the safety, tolerance and pharmacokinetics, WR 238605 was administered to 48 men in single oral doses ranging from four to 600 mg (base). It was well tolerated, with gastrointestinal disturbances as possible side effects. Linear kinetics were demonstrated at these doses. WR 238605 has a long absorption phase and is slowly metabolized, with a t_{max} of 12 hr and an elimination half-life of 14 days. These safety, efficacy and pharmacokinetic properties make this drug an excellent candidate for further testing as a prophylactic, radical curative, and terminal eradication drug.

Malaria is a leading cause of morbidity and mortality in third-world countries. About 2.4 billion people, almost half the world’s population, live in malaria-endemic areas. The annual incidence of malaria is estimated to be in the range of 100–300 million cases. Of these, approximately 1.0–1.5 million die.

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Plasmodium falciparum is considered the most important malaria parasite because of its high morbidity and mortality, especially in nonimmune individuals such as children, international travelers, and U.S. Armed Forces deployed overseas. Plasmodium vivax malaria, although less often fatal, still carries significant morbidity as well as the problem of relapses.

Primaquine is the only available drug active against exoerythrocytic (persistent tissue) stages of P. vivax or P. ovale. However, primaquine has a low therapeutic index. The most serious toxicity is acute hemolysis, which occurs in individuals who are genetically deficient in glucose-6-phosphate dehydrogenase (G6PD). Other adverse effects include abdominal pain, cramps, anorexia, nausea, vomiting, leukocytosis, leukopenia, and methemoglobinemia. Headaches, interference with visual accommodation, pruritus, hypertension, and arrhythmias have also occasionally been reported with primaquine therapy. More recently, primaquine has been shown to be better tolerated when administered with food.

However, primaquine has shown to be effective as a blood schizonticide against P. vivax parasites in nonhuman primates at doses of 0.8 and 3.2 mg/kg/day for three days. In acute oral toxicity studies, WR 238605 (50% effective dose [ED_{50}] = 0.371 μmoles/kg/day × seven days) was seven times more potent than primaquine (ED_{50} = 2.75 μmoles/kg/day × seven days). As a causal prophylactic agent, WR 238605 (ED_{50} = 0.27 μmoles/kg/day × three days) was 14 times more potent than primaquine (ED_{50} = 3.86 μmoles/kg/day × three days). Against the blood stage, WR 238605 is from four to 100 times more active than primaquine in rodent malaria models of P. falciparum malaria. It has been shown to be effective as a blood schizonticide against P. vivax and P. ovale parasites in nonhuman primates at doses of 0.8 and 3.2 mg/kg/day for three days.

In addition to its greater efficacy in both in vitro and in vivo models, WR 238605 demonstrated less toxicity than primaquine. In acute oral toxicity studies, WR 238605 (50% lethal dose [LD_{50}] = 0.78 and 0.64 mmole/kg in rats and guinea pigs) was less toxic than primaquine (LD_{50} = 0.46 and 0.12 mmole/kg). WR 238605 (WR 238605 IND # 38503) was also less toxic in subchronic and chronic studies. For example, in dog toxicology studies, 3 and 9 mg/kg/day of primaquine orally for 28 days resulted in muscle necrosis, coma, and death. Administration of WR 238605 up to the top tested dose of 16 mg/kg/day for 28 days resulted in none of these findings.

The combined greater efficacy and lower toxicity will hopefully result in a higher therapeutic index in humans. Furthermore, data from preclinical studies show that the drug has a much longer half-life than primaquine (170 versus 2 hr in the dog). These findings suggest that WR 238605 succinate has potential advantages over primaquine. We report here the results of the first human study with this new antimalarial drug.

SUBJECTS, MATERIALS, AND METHODS

Subjects. Eighty healthy adult male volunteers were recruited and signed an informed consent approved by the First Foundation for the Protection of Human Subjects in Research, Inc. (Miami Springs, FL) and the Army Surgeon General’s Human Subjects Research Review Board. Volunteers were determined to be healthy by a complete medical

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whole blood were frozen at -70–80°C until the end of the study.

**Analytical methods.** The blood and plasma samples for drug levels were analyzed by HPLC. The method used a Waters Intelligent Sample Processor (Model 710B; Waters Associates, Milford, MA), an Altex Model 100A Solvent Delivery Module (Beckman Instruments Inc., Berkeley, CA) and an Axxiom silica gel column (5 μm particle size; 4.6 × 250 mm, Richard Scientific, Novato, CA), a Hewlett-Packard Reporting Integrator #3392A (Hewlett-Packard Co., Santa Clara, CA), and a Shimadzu RF 535 Fluorescence Detector with an excitation wavelength of 375 nm and emission wavelength of 480 nm (ISI Instruspec Inc., Walnut Creek, CA). The mobile phase consisted of acetonitrile/water (1:1) with a final concentration of 5 mM (NH₄)₂HPO₄. The pH was adjusted to 7.0 with phosphoric acid.

For each plasma sample, 0.2 ml were transferred to a culture tube. Next, 10 μl of internal standard (WR 6026, 6-methoxy-8-(6-diethylaminohexylamino lepidine dihydrochloride) solution and 0.1 ml of 0.1 N NaOH buffer were added and vortexed. Three milliliters of methyl-t-butyl ether extracting solvent was then added, mixed, and centrifuged for 10 min at 3,000 × g. The organic layer was then evaporated to dryness under nitrogen. The residue was reconstituted with acetonitrile/water (1:1 [v/v]) and injected into the HPLC column with a flow rate of 1.0–1.2 ml/min. Blood samples (0.2 ml) were placed into culture tubes and 0.2 ml of nanopure water was added, vortexed, and sonicated for 10 min. Upon addition of 10 μl of internal standard, preparation followed that of plasma with the exceptions that the samples were rotated for 15 min after addition of the extracting solvent and the samples were reconstituted in methanol/water (9:1 [v/v]). Quantitation was by peak height ratio of drug relative to internal standard, and reported as ng/ml of free base. All solvents were HPLC grade; all chemicals were reagent grade. This assay has a sensitivity of 1 ng/ml for plasma and 2 ng/ml for blood, with both intra-day and inter-day coefficients of variation < 10%.

**Pharmacokinetic analysis.** Standard noncompartmental and compartmental pharmacokinetic analyses were performed. For the noncompartmental analysis, the area under the curve (AUC) was calculated by the linear trapezoidal method, with extrapolation to infinity. The terminal elimination constants were calculated using log-linear regression. For the compartmental analysis, concentration data from all subjects were simultaneously analyzed using a population approach, as well as Bayesian estimation of individual parameters. The population method allows for separate determination of both inter-individual as well as intra-individual variability. Choice of an appropriate kinetic model was made using standard goodness-of-fit criteria, such as the visual inspection of the observed and predicted concentrations and residual plots, comparison of the standard errors and confidence intervals of the parameter estimates, and values of the objective function. Any evidence of dose nonlinearity was assessed by comparison of the pharmacokinetic parameters at the different dose levels. One- and two-compartment models with and without a lag time were fit to the data.

**RESULTS**

**Demographics.** Volunteers were young, healthy adult males. The volunteers randomized to the drug and placebo
groups were comparable (Table 1). During the conduct of the study, follow-up of the 36-mg group could not be completed due to Hurricane Andrew. This dose group was repeated, and all available safety and pharmacokinetic data from both groups was included in the analyses.

Safety and tolerance. Gastrointestinal (GI) side effects (heartburn, gas, vomiting, and diarrhea) were only seen in those receiving study drug, and occurred only at higher doses (300–600 mg) (Table 2). Methemoglobinemia, hemolytic anemia, thrombocytopenia, or changes in white blood cell counts or ECGs were not observed.

Pharmacokinetics. The three volunteers receiving drug in the 4-mg group did not have samples obtained for pharmacokinetic analysis since it was not expected that their drug concentrations would be measurable. Pharmacokinetic analysis could not be performed on five other individuals, whose data was inconsistent with pharmacokinetic models, and their data was excluded from the kinetic analysis. Concentration data from the remaining 40 volunteers were analyzed. The pharmacokinetics were linear over the doses studied (Figure 2). Parameter values obtained from the non-compartmental analysis showed excellent agreement with those from the compartmental analysis. The data was best described by a one-compartment model with first-order absorption and elimination (Table 3). A proportional error model for residual, intra-individual variability was used and found to be 16.1%. Incorporation of an absorption lag time and analysis using a two-compartment model did not result in significant improvement in the corresponding objective function value. Final population parameter estimates, the standard errors of their estimates, and estimates of the inter-individual variability of each parameter estimate are shown in Table 4. The drug has a long absorption phase and is slowly cleared, with a $t_{\text{max}}$ of 12 hr and an elimination half-life of 14 days. Individual volunteer characteristics such as body weight were not found to be significant covariates for volume of distribution or clearance. Whole blood concentrations were 1.8 times higher than corresponding plasma concentrations, with resulting plasma volume of distribution and clearance values correspondingly 1.8 times higher than those of whole blood. Assuming a normal hematocrit of 45%, the drug concentration in the erythrocytes is 2.8 times that of plasma. There was no change in red blood cell accumulation over time.

**DISCUSSION**

WR 238605 is the first 8-aminoquinoline to enter clinical testing for malaria in more than 40 years. This phase I clinical study provides the first safety and pharmacokinetic data for this new primaquine analog. Based on preclinical studies, WR 238605 was much less toxic than primaquine, and in this study, WR 238605 was well tolerated. The study was designed so that the adverse event profiles and laboratory parameters could be reviewed for each lower dose before proceeding to the next higher dose. Obviously, no dose-limiting findings were encountered up to the highest dose (600 mg) studied. Side effects were few, and were not unexpected, based upon the preclinical studies and past experiences with primaquine. Like primaquine and other 8-aminoquinolines, WR 238605 may cause GI distress. Yet, while 300–600 mg of WR 238605 were associated with minimal GI side effects when administered with food, it remains to be seen if coadministration of food will reduce GI side effects of WR 238605.

Administration of primaquine to individuals with G6PD deficiency can result in serious hemolysis. The usual adult dosage of primaquine is 15 mg (26.3 mg of primaquine phosphate) a day for 14 days. Alternatively, adults may receive 45 mg of primaquine (79 mg of primaquine phosphate) once a week for eight weeks. This weekly regimen has been demonstrated to be therapeutically effective in the radical

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**TABLE 1**

Demographic data of the study subjects*

<table>
<thead>
<tr>
<th>Drug (n = 48)</th>
<th>Placebo (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>27.8 ± 4.7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76.1 ± 8.2</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>176.4 ± 7.3</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>20 (42%)</td>
</tr>
<tr>
<td>Black</td>
<td>12 (25%)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>16 (33%)</td>
</tr>
</tbody>
</table>

* Values are the mean ± standard deviation.

**TABLE 2**

Adverse experiences* rated as possibly or probably related to treatment

<table>
<thead>
<tr>
<th>Experience</th>
<th>Active (n = 48)</th>
<th>Placebo (n = 32)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Headache</td>
<td>2 (288, 300)</td>
<td>2 (100, 240)</td>
</tr>
<tr>
<td>Lightheaded, dizzy</td>
<td>3 (36, 350, 350)</td>
<td>1 (350)</td>
</tr>
<tr>
<td>Heartburn, gas</td>
<td>4 (300, 300, 600)</td>
<td>0</td>
</tr>
<tr>
<td>Vomiting</td>
<td>1 (600)</td>
<td>0</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1 (500)</td>
<td>0</td>
</tr>
<tr>
<td>Fever</td>
<td>0</td>
<td>2 (192, 192)</td>
</tr>
<tr>
<td>Microvesicular rash</td>
<td>0</td>
<td>1 (288)</td>
</tr>
<tr>
<td>Eye irritation</td>
<td>1 (16)</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>6</td>
</tr>
</tbody>
</table>

* Values are the number of events (dose groups at which events occurred). All events were mild in severity.
cure of *P. vivax* without producing clinical hemolysis in individuals with the A-variant of G6PD deficiency. WR 238605 has not been administered to humans with this deficiency. This drug may produce hemolysis in deficient individuals, but the relative hemolytic potential of WR 238605 compared with primaquine is unknown. A study to evaluate the safety of WR 238605 in G6PD-deficient individuals is planned.

The absorption half-life (t_{1/2abs}) of 1.7 hr suggests rapid absorption. However, the time to peak concentration (t_{max}) of 13.8 hr implies prolonged absorption from the gut. Dissolution studies of WR 238605 in simulated gastric fluid demonstrate complete dissolution within 30 min. Thus, the long apparent absorption phase may be due to a distal GI absorption site combined with the drug’s slow clearance since t_{max} is a function of both absorption and elimination rates. Primaquine is well absorbed from the gastrointestinal tract with peak plasma levels attained within 1–6 hr.

The apparent volume of distribution (V/f) of WR 238605 was large (2,550 L). Although we do not know the absolute bioavailability in humans, in beagle dogs it was found to be 60–100% (Hawkins DR, Huntingdon Research Centre, Ltd., Huntingdon, Cambridgeshire, United Kingdom). Even if the bioavailability in humans is only 60%, that would make the true volume of distribution approximately 1,530 L. This is still a large volume of distribution, and suggests extensive tissue binding.

In preclinical studies, WR 238605 was found to be slowly but extensively metabolized. The drug is eliminated via biliary excretion with enterohepatic recirculation, but is not eliminated in the urine (Hawkins DR, Huntingdon Research Centre, Ltd., unpublished data). Primaquine is similarly extensively and rapidly metabolized with less than 1% of the dose excreted as unchanged drug in urine. In this study, the clearance (CL/f) of WR 238605 was only 4.7 L/hr (<5% of the hepatic blood flow), while primaquine has a much more rapid clearance of 30 L/hr.

WR 238605 was found in this study to have a half-life that is more than 50 times longer than primaquine. Figure 3 illustrates the effect that this difference in half-lives between these two 8-aminoquinolines has. A single 600-mg dose of WR 238605 results in easily quantifiable concentrations for more than five weeks, whereas 14 daily 15-mg doses of primaquine result in no detectable concentrations 1–2 days after the last dose. Other antimalarial drugs with long half-lives (mefloquine, halofantrine, chloroquine) are best described by multicompartment models, with central compartment concentrations decreasing rapidly after the peak due to a rapid distribution phase. WR 238605, on the other hand, in this study was best described by a one-compartment model. This kinetic difference results in more prolonged, high concentrations of the antimalarial drug in the blood. These properties may be an advantage over primaquine in that they may permit weekly dosing for prophylaxis and short-term or single dose therapy for terminal eradication or radical cure of *P. vivax* malaria, perhaps resulting in improved compliance and enhanced effectiveness.

Standard errors of all pharmacokinetic parameter estimates were low (coefficient of variation [CV] = 10%) with low intra-individual (residual) variability (16.1%), providing confidence in the model. There was moderate inter-individual variability in V/f and CL/f. However, as is often the case with water-insoluble drugs, a great deal of variability in the
absorption rate constant (CV = 57.8%) was found. Co-administration with food or drug re-formulation may reduce this variability, and further studies to address this remain to be done. Individual demographic characteristics such as age or weight did not have significant relationships with the pharmacokinetics in this study, although this was a very select, healthy population with normal liver function and a narrow weight and age range.

WR 238605 has generally been found to be much more potent than primaquine as a blood schizonticide. This may be explained in part by its accumulation inside the red blood cells and its longer half-life, in addition to any difference in intrinsic activity. Primaquine, on the other hand, does not accumulate inside erythrocytes.20-22 Primaquine has recently been shown to have efficacy as a prophylactic drug against *P. falciparum* malaria,20 and studies evaluating WR 238605 for this indication are in progress. Given the greater antimalarial activity, its greater erythrocytic accumulation, and its longer half-life, WR 238605 is a promising candidate, both as a weekly administered prophylactic drug as well as a short regimen treatment for *P. vivax* malaria. Additional studies to evaluate the safety and pharmacokinetics in other populations, the effects of food on absorption, and its efficacy are under way.

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