Atovaquone and Proguanil Hydrochloride: A Review of Nonclinical Studies

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Background: Safe and effective antimalarial drugs are needed for treatment and prophylaxis of malaria. The combination of atovaquone and proguanil hydrochloride is a new antimalarial drug combination that has recently become available in many countries.

Methods: Data were reviewed from nonclinical studies evaluating the microbiology, secondary pharmacology, pharmacokinetics, and toxicology of atovaquone and proguanil hydrochloride.

Results: Atovaquone is highly active against asexual erythrocytic stages of Plasmodium falciparum in vitro (IC_{50} 0.7–6 nM) and in animal models. Proguanil per se has only weak antimalarial activity in vitro (IC_{50} 2.4–19 uM), and its effectiveness depends on the active metabolite cycloguanil (IC_{50} 0.5–2.5 nM). The combination of atovaquone and proguanil is synergistic in vitro. Both drugs also have activity against gametocytes and pre-erythrocytic (hepatic) stages of malaria parasites. Atovaquone is a ubiquinone antagonist that inhibits mitochondrial electron transport and collapses mitochondrial membrane potential. The proguanil metabolite cycloguanil is a dihydrofolate reductase inhibitor, but the mode of action of proguanil is unknown. In screening evaluations of secondary pharmacology, neither atovaquone nor proguanil had activity that adversely affected gastrointestinal, cardiovascular, or central or autonomic nervous system functions or clinically relevant concentrations. After oral administration, atovaquone exposure is extensive in rats but limited in dogs, while proguanil and cycloguanil exposure is extensive in dogs but limited in rats. In both species, toxicity was related to proguanil exposure, the principal manifestations being salivation, emesis, and loss of body weight. Neither atovaquone nor proguanil was teratogenic or mutagenic. An increased incidence of hepatic adenomas and adenocarcinomas was seen in mice, but not rats, after lifetime exposure to atovaquone, and appears to be related to species-specific differences in hepatic enzymatic activity. No additional toxicity was evident in animals treated with the combination of atovaquone and proguanil hydrochloride compared to those treated with either drug alone.

Conclusion: Nonclinical studies of atovaquone and proguanil hydrochloride supported the clinical development of this combination for treatment and prophylaxis of malaria.

The continuing spread of drug-resistant malaria and concerns about the safety of antimalarial drugs, particularly when used for prophylaxis, emphasize the need for new approaches to antimalarial therapy, especially the discovery and development of drugs with novel modes of action. The combination of atovaquone and proguanil hydrochloride (Malarone) has recently been approved for treatment and prophylaxis of malaria. In conjunction with clinical studies of this new antimalarial drug combination, an extensive series of nonclinical studies were conducted with atovaquone and proguanil hydrochloride, both separately and in combination. In this paper, we review data from the microbiology, secondary pharmacology, toxicology and pharmacokinetics, and absorption, distribution, metabolism, and excretion (ADME) studies of atovaquone and proguanil hydrochloride conducted to support the development of a fixed-dose combination tablet for treatment and prevention of malaria.

Materials and Methods

We reviewed published literature and unpublished data that provide information on nonclinical evaluations of atovaquone or proguanil hydrochloride. This included studies of the primary pharmacology (microbiology), both in vitro and in animal models of malaria, secondary (safety) pharmacology (i.e., physiologic activity unrelated to antimalarial effects), pharmacokinetics, and toxicology.
Results

Microbiology

Atovaquone (Figure 1) is a hydroxynaphthoquinone with potent antimalarial activity. In vitro, the IC₅₀, against sexual erythrocytic stages of wild type isolates ranges from 0.7–6 nM.¹ It is also effective in rodents infected with various drug-resistant strains of Plasmodium yoelii and P. berghei, and cures P. falciparum infections in Aotus monkeys.¹ Atovaquone also has cause prophylactic activity against the pre-erythrocytic (hepatic) stages of P. berghei.²⁻⁵ It inhibits early gametocytes of P. falciparum,⁶ inhibits ookinete formation from mature P. berghei gametocytes in vitro,⁷ and inhibits sporogonic stages of P. berghei gametocytes in mosquitoes.⁸

The mechanism of action of atovaquone against P. falciparum is via inhibition of mitochondrial electron transport.⁹ Atovaquone has a novel mode of action, inhibiting the electron transport system at the level of cytochrome bc₁ complex. In malaria, there is an obligatory coupling of pyrimidine biosynthesis and electron transport via ubiquinone/ubiquinol. Selectivity is achieved by virtue of the different sensitivities of mammalian and plasmodium electron transport systems to the hydroxynaphthoquinones (thousandfold difference); thus, side effects are limited. Moreover, plasmodium is totally reliant on pyrimidine biosynthesis, whereas mammalian cells are able to salvage pyrimidines. Atovaquone also causes collapse of the parasite mitochondrial membrane potential in P. yoelii¹⁰ and P. falciparum (A. Vaidya, personal communication). Parasites highly resistant to atovaquone can be selected by drug pressure in vitro¹¹ or by treatment of symptomatic P. falciparum infections with atovaquone alone.¹² Drug resistance appears to be associated with single point mutations in the cytochrome b gene.¹¹

Proguanil (Figure 2) is a biguanide that is metabolized to cycloguanil and, to a lesser extent, to 4-chlorophenyl biguanide. Against sexual erythrocytic stages of P. falciparum, cycloguanil has potent activity (IC₅₀ 18–36 nM in standard culture medium)¹³ and 0.5–2.5 nM in medium deficient in folic acid and p-aminobenzoic acid), while proguanil (IC₅₀ 2.4–19 μM) and 4-chlorophenyl biguanide (IC₅₀ 11–40 μM) have weak but measurable activity.¹⁴ Proguanil has activity against both the erythrocytic and pre-erythrocytic (hepatic) stages of P. berghei in mice¹⁵ and P. falciparum in man.¹⁶ Cycloguanil is active against the hepatic stages of P. yoelii in vitro.¹⁶ Proguanil decreases infectivity of P. falciparum gametocytes¹⁷ and inhibits P. falciparum and B. vivax oocyst development in mosquitoes.¹⁸

The mechanism of action of proguanil, via its metabolite cycloguanil, is inhibition of dihydrofolate reductase (DHFR). The affinity of malarial DHFR from P. berghei (Ki = 0.8 nM) is several hundredfold stronger than the mouse erythrocyte DHFR, thus documenting the selective toxicity of cycloguanil on an enzymatic basis. Much higher concentrations of proguanil (200 μM) do not inhibit DHFR from P. berghei, rat liver, or Escherichia coli. Plasmodia synthesize folate cofactors de novo and cannot use intact exogenous folates. Therefore, selective inhibition of the parasite DHFR will result in depletion of tetrahydrofolate cofactors required for cellular metabolism, especially DNA synthesis, and thus prevent growth.¹⁸ Mutations in the DHFR gene are associated with resistance to cycloguanil.¹⁷ Proguanil also appears to have antimalarial activity independent of its metabolism to cycloguanil. Cycloguanil-resistant parasites retain their sensitivity to high concentrations of proguanil.¹⁷ P. falciparum transformed with a variant form of human DHFR selectable by methotrexate has greater resistance to cycloguanil but no decrease in the level of susceptibility to proguanil, thus providing direct evidence of intrinsic activity of this parent compound against a target other than DHFR.¹⁸ The additional mechanism of action of proguanil is not well defined but may involve mitochondrial toxicity, since proguanil, but not cycloguanil, is able to potentiate the ability of atovaquone to collapse mitochondrial membrane potential in P. yoelii (A. Vaidya, personal communication). The antimalarial action of 4-chlorophenyl biguanide may be similar.

Synergistic activity between atovaquone and both proguanil and cycloguanil has been demonstrated in
vitro against the erythrocytic stages of *P. falciparum*.\(^{22}\) This observation formed the microbiologic basis for the clinical development of atovaquone and proguanil hydrochloride as a fixed combination.\(^{12}\)

**Secondary Pharmacology**

The secondary pharmacology of atovaquone and proguanil hydrochloride was evaluated for the separate compounds in rats, mice, and dogs, and the combination was evaluated in conscious Beagle dogs.\(^{11}\) Systemic exposure to plasma concentrations of atovaquone, proguanil, and cycloguanil within the therapeutic range in humans is well tolerated. In the animal models used, there are no significant effects on the cardiovascular, respiratory, or central or autonomic nervous systems.

**ADME Studies**

After oral administration, atovaquone is poorly absorbed in all species studied (mice, rats, rabbits, and dogs). Increases in plasma concentrations with increasing doses are less than proportional, and the absolute bioavailability does not exceed 51% at low doses or 5% at the highest doses tested. In dogs given 20 mg/kg, average \(C_{\text{max}}\) values are 1.7 \(\mu\)g/mL fasting and 6.3 \(\mu\)g/mL with food. The elimination half-life averages ~24 h in mice, ~26 h in rats, ~22 h in rabbits, and ~50 h in dogs and is independent of dose. Protein binding is extensive (>99%) in all species studied and is unaffected by the presence of other compounds, including proguanil. Distribution into tissues after absorption is limited to the organs of excretion and metabolism. Hepatic P450 enzymes of the 2B family are induced in mice, but not in rats, by atovaquone. Atovaquone does not appear to induce its own metabolism; biotransformation is not a significant factor in the disposition of the compound. More than 90% of an administered dose is recovered as unchanged drug in feces, and renal elimination is negligible (<3%).\(^{11}\)

Absorption of proguanil hydrochloride after oral administration is fairly complete in mice, rats, rabbits, and monkeys.\(^{23-24}\) At 40 mg/kg/d, average \(C_{\text{max}}\) values for proguanil are 0.22 \(\mu\)g/mL in rats and 2.1 \(\mu\)g/mL in dogs.\(^{11}\) The elimination half-life ranges from 1 to 5 h and shows no evidence of accumulation after repeated administration. Protein binding is 65% for rabbit plasma, 75% for human plasma, and 81% for rat and dog plasma.\(^{11}\) The metabolism of proguanil to cycloguanil is high in rabbits, dogs, and monkeys and low in rats. At 40 mg/kg/d, average \(C_{\text{max}}\) values for cycloguanil are <0.05 \(\mu\)g/mL in rats and 0.17 \(\mu\)g/mL in dogs. Proguanil does not induce its own metabolism after repeated administration to rats, and there is no evidence for autoinduction in other species. The major route of elimination is renal excretion in dogs and fecal excretion in rats. In vitro studies with human liver microsomes indicate cycloguanil can be formed by human P450 enzymes 2C19 and 3A4.\(^{15}\) Recent data have confirmed that CYP2C19 is the major enzyme responsible for proguanil bioactivation.\(^{26}\)

Administration of atovaquone and proguanil hydrochloride in combination does not affect the pharmacokinetics of either drug compared to the pharmacokinetics when given separately.\(^{11}\) Systemic exposures to atovaquone, proguanil, and cycloguanil in rats and dogs during long-term toxicity studies brackets those seen in clinical studies of atovaquone and proguanil hydrochloride for malaria prophylaxis in humans (Table 1).

**Toxicology**

**Acute Toxicity.** The acute oral \(LD_{50}\) of atovaquone in mice and rats is in excess of 1825 mg/kg (the highest dose tested). No deaths or other treatment-related effects are seen.\(^{11}\) The acute oral \(LD_{50}\) of proguanil hydrochloride is 23, 200, 130, >400 (no deaths), and >400 (no deaths) in the mouse, rat, rabbit, dog, and monkey, respectively.\(^{23,24}\)

**Repeat-dose Toxicity.** Repeat-dose oral toxicity studies with atovaquone have been carried out in mice for up to 90 days (maximum dose 800 mg/kg/d) and in rats and dogs for up to 12 months (maximum dose 500 mg/kg/d).\(^{11}\) At the highest dose in mice, liver toxicity is manifest by hepatocellular hypertrophy and individual cell necrosis. In rats, minor variations in hematologic parameters are seen that are reversible on cessation of treatment. Concentrations of atovaquone in plasma increase with dose but proportionally, to an average maximum of approximately 78 \(\mu\)g/mL. In dogs, no treatment-related effects are seen, except yellow feces and pink plasma, which are attributed to the presence of the drug. Average plasma concentrations reach a maximum of approximately 23 \(\mu\)g/mL at 500 mg/kg/d. The liver toxicity seen in mice is not seen in either rats or dogs.

In repeat-dose oral toxicity studies with proguanil hydrochloride, doses of 9 to 32 mg/kg/d for 10 days in mice cause decreased food consumption and body-weight gain. In rats, decreases in food consumption and body-weight gain were observed at doses of 9 mg/kg/d. In monkeys, decreases in food consumption and body-weight gain were observed at doses of 3 mg/kg/d.

**Table 1** Comparison of Systemic Exposure (AUC) to Atovaquone, Proguanil, and Cycloguanil in Animals and Humans After Oral Administration of Atovaquone and Proguanil Hydrochloride

<table>
<thead>
<tr>
<th>Dose (mg/kg/day)</th>
<th>Atovaquone</th>
<th>Proguanil</th>
<th>Cycloguanil</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat</td>
<td>50:20</td>
<td>1477</td>
<td>0.92</td>
</tr>
<tr>
<td>Dog</td>
<td>30:12</td>
<td>86</td>
<td>8.77</td>
</tr>
<tr>
<td>Human</td>
<td>5:21</td>
<td>50</td>
<td>0.64</td>
</tr>
</tbody>
</table>

*BLQ = below limits of quantitation.

1Assuming 250 mg atovaquone and 100 mg proguanil hydrochloride per day in a 50 kg person.
weight gain, and a dose of 45 mg/kg/day for 10 days is lethal.\textsuperscript{22} Rats given 40 mg/kg/d for 2 months have a slight decrease in body weight, and a dose of 50 mg/kg/day causes deaths, but without pathologic correlates.\textsuperscript{23} At 20 mg/kg/d for 6 months, changes are limited to decreased bodyweight and microscopic changes in the kidney (tubular basophilia) and cecum (mucosal hyperplasia) that are reversible.\textsuperscript{11} In dogs, daily oral doses of 20 to 160 mg/kg/d for 2 months cause copious salivation, emesis, loss of appetite and body weight, extreme emaciation, hyperconcentration, death, increased liver weight, and hyperemic gastric mucosa. There is no dose-related pathologic correlate, and these toxicities are fully reversible when treatment is stopped after animals become critically ill.\textsuperscript{11} At moderate doses, proguanil hydrochloride is better tolerated in monkeys than in dogs, although similar effects occur at high dose levels.\textsuperscript{24} At a dose of 12 mg/kg/d for 6 months in dogs, proguanil hydrochloride causes bile duct hyperplasia, gall bladder mucosal atrophy, and fibrovascular proliferation in the right atrium.\textsuperscript{11}

Treatment with a fixed combination of atovaquine:proguanil hydrochloride for 6 months in rats (50:20 mg/kg/d) and dogs (30:12 mg/kg/d) does not produce any effects not seen with the individual drugs alone. \textit{Reproductive Toxicity}. Neither atovaquine nor proguanil hydrochloride is teratogenic. Atovaquine is not embryotoxic to rat fetuses (at doses up to 1000 mg/kg/d) or to rabbit fetuses (doses up to 1200 mg/kg/y). There is no effect on the incidence of malformations or variations in either species. At doses of up to 1000 mg/kg/d, atovaquine has no adverse effects on fertility, general reproductive performance, or perinatal development in the rat.\textsuperscript{11} Administration of proguanil hydrochloride to mice prior to mating and during early gestation causes reduced fertility.\textsuperscript{25} Oral administration of cyclophosphamide on the first day of gestation in rats produces a high incidence of fetal deaths, while oral administration from days 8 to 13 has no effects.\textsuperscript{26} Oral administration of atovaquine and proguanil hydrochloride during pregnancy in a fixed ratio combination (2:5:1) up to 50:20 mg/kg/d in the rat and up to 100:40 mg/kg/d in the rabbit, is not embryotoxic and has no effect on the nature or incidence of fetal variations or malformations.\textsuperscript{11}

\textit{Mutagenesis and Carcinogenesis}. Both atovaquine and proguanil are negative with or without metabolic activation in the Ames Salmonella mutagenicity assay, the mouse lymphoma mutation assay, and the cultured human lymphocyte cytogenetic assay. No evidence of genotoxicity was observed in the in vivo mouse micronucleus assay with either compound.\textsuperscript{11}

In a 2-year oral oncogenicity study in mice, an increase in the incidence of hepatocellular adenomas and carcinomas was seen. These occur in a mouse strain that has a high historic incidence of spontaneous liver tumors, and there is no relationship between tumor incidence seen in this study with either dosage or systemic exposure to atovaquine. In a 2-year oral oncogenicity study in rats, there were no treatment-related increases in the incidence of benign or malignant tumors in any tissue or organ.\textsuperscript{11} Oncogenicity studies with proguanil hydrochloride have not been completed.

\section*{Discussion}

The mode of action of atovaquine is unique among currently available antimalarial drugs, which explains its activity against parasite strains resistant to all other registered drugs. Extreme selectivity at the target site and the ability of the host, but not the parasite, to use pyrimidine salvage pathways contribute to its excellent safety profile. Because single point mutations can result in loss of susceptibility, atovaquine must be used in combination with another antimalarial drug. The excellent clinical results with atovaquine and proguanil hydrochloride,\textsuperscript{27} including efficacy in patients who are poor proguanil metabolizers and who are infected with multi-drug resistant parasites, indicate that the synergy seen in vitro between atovaquine and proguanil, cyclophosphamide, and 4-chlorophenyl biguanide must extend to the in vivo situation. The causal prophylactic activity and possible transmission-blocking ability of both atovaquine and proguanil may reduce the rate at which clinically important resistance to this combination will develop.

There is a moderate degree of interspecies variability in the pharmacokinetics of atovaquine and proguanil hydrochloride in animals. Compared to plasma drug concentrations during clinical studies in humans, plasma concentrations in rats are higher for atovaquine, similar for proguanil, and negligible for cyclophosphamide, reflecting the fact that rats are poor metabolizers of proguanil. In dogs, plasma concentrations of atovaquine are similar and concentrations of proguanil and cyclophosphamide are higher than those seen in humans.

In both rats and dogs, the maximum dose of the atovaquine and proguanil hydrochloride combination that can be administered is limited by the gastrointestinal toxicity of the proguanil component. Atovaquine is also used for treatment and prevention of \textit{Plasmodium carini} pneumonia and toxoplasmosis,\textsuperscript{28} and previous nonclinical evaluations have demonstrated that administration of much higher doses of atovaquine produce little toxicity in mice, rats, and dogs.

Oncogenicity studies of atovaquine in rodents show an increased incidence of liver tumors (adenomas and carcinomas) in mice, but not in rats, treated with atovaquine for 2 years. In 3-month studies of atovaquine, hepatic effects (increases in liver weight, hepatocellular hypertrophy, proliferation of smooth endoplasmic reticulum, individual cell necrosis, and focal necrosis) are
observed in mice, but not in rats. In vitro and in vivo genetic toxicity studies are negative. These results are consistent with species-specific hepatic enzyme induction. Atovaquone induces cytochrome P450 2B1 in mice but not rats, and the equivalent isoenzyme in humans (P450 2B6) is expressed at very low levels in normal human livers. Induction of this enzyme has been implicated as a mechanism of mouse-specific hepatic tumorigenesis by other drugs and chemicals. These findings appear to be due to the inherent susceptibility of mice to atovaquone and are not predictive of a risk in the clinical situation.

In summary, dosing of animals with atovaquone and progama hydrochloride is limited by gastrointestinal toxicity related to the progama component. There is no additional toxicity seen with the combination that is not predicted from studies with the individual components, and neither drug is considered to pose a significant genetic risk. Thus, nonclinical studies of atovaquone and progama hydrochloride support the clinical development of this combination for treatment and prophylaxis of malaria.

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References


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