# CENTER FOR DRUG EVALUATION AND RESEARCH

*APPLICATION NUMBER:*

**21-078**

# PHARMACOLOGY REVIEW

**NDA 21-078**
**Date Submitted:** 29 December 1998
**Date Assigned:** 5 January 1999
**Date Completed:** 11 June 1999

**Related NDAs:** 20-259
**Related INDs:** [redacted]
**HFD-590**

**Sponsor:** GlaxoWellcome
Research Triangle Park, NC 27709

**Drug:** atovaquone (566C80; 566C); 2-[trans-4-(4-chlorophenyl) cyclohexyl]-3-hydroxy-1,4-naphthoquinone and proguanil HCl (336U50); 1-(4-chloropheny;0-5-isopropyl biguanide hydrochloride in a 2.5:1 ratio

**Formulation:** Malarone tablets, 250 mg atovaquone/100 mg proguanil; pediatric tablets, 62.5 mg atovaquone, 25 mg proguanil

## INTRODUCTION

Malarone is a fixed combination of an approved and marketed product, atovaquone (Mepron™) and a non-US marketed product, proguanil (Paludrine), in a ratio of 2.5:1. Atovaquone has been in use since USFDA approval in 1992. Originally developed as an antimalarial agent, the label indication for atovaquone is *Pneumocystis carinii* infection in immunocompromised patients, especially with AIDS. Proguanil has been used as an antimalarial agent abroad since the 1940s. Proguanil was submitted to the FDA in the 1960s but withdrawn by the sponsor. Human experience with each of these agents has demonstrated low incidence of adverse events.

Resistance to antimalarial drugs has become a critical issue in development of new agents and therefore many are developed for use in combination treatment. Combination treatments are advantageous for preventing emerging resistance, particularly if agents of differing mechanism of action are employed. Atovaquone acts as a potent and selective inhibitor of the mitochondrial transport chain in susceptible protozoa, inhibiting pyrimidine synthesis. Proguanil acts as an inhibitor of dihydrofolate reductase (DHFR), blocking folate metabolism in *Plasmodium*.

The studies submitted to this NDA appear to be directed to elucidate pharmacokinetic and toxicological properties of proguanil, as well as to show combination effects with atovaquone. Atovaquone pharmacology, toxicology, genetic toxicology and reproductive toxicology have been reviewed under NDA 20-259 and relevant INDs (attached).

## PHARMACOLOGY

Proguanil inhibits dihydrofolate reductase-thymidylate synthetase, leading to depletion of the tetrahydrofolate cofactors that are required in the synthesis of purines and thymidilate through the formation of a cyclic triazine metabolite, cycloguanil. Cycloguanil inhibits *Plasmodium* DHFR at concentrations much lower than those which inhibit mammalian DHFRs. Studies referring to the antimalarial mechanism of action will be found in the microbiologist's review.

**Pharmacology Study**

| Study Title: **Cardiovascular and behavioral effects of the anti-malarial agents, atovaquone and proguanil HCl, following their oral administration, either separately or in combination (2.5:1 ratio), to the conscious beagle dog** | Study No: BBIO/95/0019/00 | GLP compliance Yes (x) No () |
|---|---|---|
| Conducting laboratory and location: The Wellcome Foundation Beckenham, UK | Date of study finalization: 31 August 1995 | QA- Report Yes (x) No () |

Six conscious non-naive beagle dogs, 1/dose/sex, were administered an oral dose of either atovaquone, 20 mg/kg, atovaquone and proguanil, 20 and 8 mg/kg, respectively, or vehicle control. Cardiovascular parameters, arterial blood gases and acid/base balance were monitored for 24h. Possible central nervous and autonomic nervous system dysfunctions and drug plasma levels were monitored for 14 days following dosing. Arterial blood pressure and cardiac conductivity were unaffected by drug treatment. Occasional emesis occurred within 1-2 h of dosing. Otherwise no behavioral effects occurred. Blood gas and acid/base status were unaffected by treatment. Clinical chemistry and hematology were unaffected by treatment. The value of this study is limited by the small group size.

## NONCLINICAL PHARMACOKINETICS

**Rat pharmacokinetic studies**

The pharmacokinetics of atovaquone and proguanil in the male and female Hsd:Sprague Dawley rat following single oral administration of $^{14}$C-proguanil in combination with atovaquone, at dose levels of 8mg/kg and 20mg/kg

A toxicokinetic study in pregnant rats given 566C80 (atovaquone) and 336U50 (proguanil HCl) in 2.5:1 ratio by gavage

A whole body autoradiography study in male Sprague Dawley rats following oral administration of a combination of atovaquone at a dose of 20 mg/kg and $^{14}$C-proguanil at a dose of 8mg/kg

A whole body autoradiography study in time mated Sprague-Dawley rats following oral administration of a combination of atovaquone at a dose of 12.5 mg/kg and proguanil at a dose of 5 mg/kg

Radioprofiling of plasma, urine and fecal extracts following oral administration of $^{14}$C-proguanil (8mg/kg) combined with atovaquone (20mg/kg) to the rat

A study to investigate the effects upon hepatic drug metabolizing enzyme activity in male Sprague Dawley rats following daily oral administration of atovaquone and proguanil in the ratio 2.5:1

The rates and routes of excretion of radioactivity from male Sprague Dawley rats after oral administration of $^{14}$C-proguanil in combination with atovaquone at doses of 8mg/kg and 20mg/kg respectively

**Rabbit Pharmacokinetic Study**
Toxicokinetic study in pregnant rabbits given 566C89 (atovaquone) and 336U50 (proguanil HCl) in 2.5:1 ratio by gavage

**Dog Pharmacokinetic Studies**
The pharmacokinetics of atovaquone and proguanil in the male and female beagle dog following single oral administration of $^{14}$C-proguanil alone, or $^{14}$C-proguanil in combination with atovaquone, at dose levels of 6mg/kg proguanil and 15mg/kg atovaquone

The rates and routes of excretion of radioactivity from male and female beagle dogs following single oral administration of $^{14}$C-proguanil in combination with atovaquone, at dose levels of 6mg/kg proguanil and 15mg/kg atovaquone

***In vitro* Pharmacokinetic Studies**
Study of protein binding of proguanil and to investigate potential displacement from binding sites on plasma proteins in rat, dog and human plasma in the presence of atovaquone

A study of the metabolism in vitro of proguanil using hepatic microsomes prepared from rat, rabbit, dog, monkey and human tissue

A study to investigate the in vitro metabolism of proguanil using hepatocytes isolated from rat, rabbit, dog, cynomologus monkey and human

A study to investigate the potential for the glucuronidation of atovaquone by hepatic enzyme preparations from animals and man

**Pharmacokinetic Studies**

| Study Title: **The pharmacokinetics of atovaquone and proguanil in the male and female Hsd:Sprague Dawley rat following single oral administration of $^{14}$C-proguanil in combination with atovaquone, at dose levels of 8mg/kg and 20mg/kg** | Study No: BDRR/94/0035 | GLP compliance Yes (x) No () |
|---|---|---|
| Conducting laboratory and location: Burroughs Wellcome Co. 3030 Cornwallis Rd. Research Triangle Park, NC 27709 | Date of study finalization: 13 June 1995 | QA- Report Yes (x) No () |

Sprague Dawley rats (3/sex/group/phase) were given atovaquone, 20 mg/kg and proguanil, 8 mg/kg, and $^{14}$C-proguanil (batch CA, specific activity 113mCi/mmol, and batch CB, specific activity 99.6 mCi/mmol) in 0.25% methyl cellulose in a single oral dose. Blood samples were collected at 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 32, 48, 72 and 96h after dosing. Blood and plasma samples were assayed for radioactivity by [redacted] Plasma samples were analyzed for drug concentrations by [redacted] and pharmacokinetics determined. Atovaquone Cmax occurred at 16h after dosing, with mean Cmax of 24.95 µg/ml for males and 31.15 µg/ml for females. $AUC_{0-\infty}$ were 943 µg/ml.h for males and 1513 µg/ml.h for females. Proguanil and/or metabolites were only detected in two samples by [redacted] quantitation by [redacted] displayed maximum levels between one and three hours following dosing. Higher levels of radioactivity were seen in females.

| Study Title: **A toxicokinetic study in pregnant rats given 566C80 (atovaquone) and 336U50 (proguanil HCl) in 2.5:1 ratio by gavage** | Study No: TTEP/95/0067 | GLP compliance Yes (x) No () |
|---|---|---|
| Conducting laboratory and location: Burroughs Wellcome Co. 3030 Cornwallis Rd. Research Triangle Park, NC 27709 | Date of study finalization: 22 January 1996 | QA- Report Yes (x) No () |

Female CD rats ([redacted] were assigned 20/group to the following groups: (atovaquone:proguanil mg/kg) 12.5:5, 25:10, 50:20, 50:0, and 0:20. Rats were then mated. Daily drug treatment followed on days 6 through 17 of gestation based on the mating date. On day 27, blood was collected from the dams (3/group/timepoint) at 0.5, 1, 2 and 4h postdosing. Plasma concentrations of atovaquone and proguanil were determined by [redacted] for calculation of pharmacokinetic values.

| | 12.5:5 mg/kg atovaquone: proguanil | 25:10 mg/kg atovaquone: proguanil | 50:20 mg/kg atovaquone: proguanil | 50:0 mg/kg atovaquone: proguanil | 0:20 mg/kg atovaquone: proguanil |
|---|---|---|---|---|---|
| Atovaquone AUC (µg/ml.h) | 436.5 | 754.4 | 945.6 | 660.1 | |
| Atovaquone Cmax (µg/ml) | 30.81 | 58.89 | 72.02 | 48.49 | |
| Proguanil AUC (µg/ml.h) | Not estimated | Not estimated | 0.593 | | 0.447 |
| Proguanil Cmax (µg/ml) | <0.04 | 0.062 | 0.094 | | 0.105 |

Exposure to atovaquone may have been increased by coadministration with proguanil. At the 50:20 mg/kg dose, AUC and Cmax were slightly higher than in the 50:0 mg/kg group. As the 50:20 mg/kg dose was the only dose which provided pharmacokinetic information (AUC) on proguanil due to the apparent lack of exposure at lower doses, a human equivalent dose would be a dose of 8.33/3.33 mg/kg.

| Study Title: **A whole body autoradiography study in male Sprague Dawley rats following oral administration of a combination of atovaquone at a dose of 20 mg/kg and $^{14}$C-proguanil at a dose of 8mg/kg** | Study No: BDRR/94/0037 | GLP compliance Yes (x) No () |
|---|---|---|
| Conducting laboratory and location: Wellcome Research Laboratories Beckenham, Kent, UK | Date of study finalization: 23 August 1995 | QA- Report Yes (x) No () |

Sprague Dawley rats (4 males, [redacted] were given atovaquone, 20 mg/kg (batch Q4M) and proguanil (batch P579R), 8 mg/kg, and $^{14}$C-proguanil (batch CA, specific activity 113 mCi/mmol) in 0.25% methyl cellulose by oral gavage. Two were sacrificed at 1h and 24h after dosing. Carcasses were frozen for subsequent sectioning and autoradiography.

At 1h post dosing, highest radioactivity was found in the stomach and intestinal contents. The only tissue with radioactivity was the liver (3.06 µg equivalents/g). At 24h post dosing, radioactivity was greatest in the liver (0.96 µg equivalents/g) and intestinal contents, with lesser amounts present in the cortico-medullary junction of the kidney (1.14 µg equivalents/g) and the brown fat (0.35 µg equivalents/g). At both timepoints, the liver exhibited a nonheterogenous, or speckled, pattern of radioactivity in autoradiographs. It appears through 24h following dosing that proguanil does not distribute well to the tissues of the rat.

| Study Title: **A whole body autoradiography study in time mated Sprague-Dawley rats following oral administration of a combination of atovaquone at a dose of 12.5 mg/kg and proguanil at a dose of 5 mg/kg** | Study No: BDRR/95/0017 | GLP compliance Yes (x) No () |
|---|---|---|
| Conducting laboratory and location: Burroughs Wellcome Co. 3030 Cornwallis Rd. Research Triangle Park, NC 27709 | Date of study finalization: 30 January1996 | QA- Report Yes (x) No () |

Sprague Dawley rats (6 females, time-mated for gestation day 11, [redacted], were acclimated one week and then given atovaquone, 20 mg/kg (batch Q4M) and proguanil (batch P579R), 8 mg/kg, and $^{14}$C-proguanil (batch CA, specific activity 113 mCi/mmol) in 0.25% methyl cellulose by oral gavage. Two rats/timepoint were sacrificed at 1, 4 and 24h after dosing. Carcasses were frozen for subsequent sectioning and autoradiography. At one hour post dosing, the greatest amount of radioactivity was found in the stomach and intestinal contents. In tissues, radioactivity was found in the liver (2.39 µequivalents/g) and bone (0.80 µequivalents/g in vertebrae, 13.09 µequivalents/g in the skull). At 4 hours post dosing, the greatest amount of radioactivity was again found in the stomach and intestinal contents. Tissue radioactivity was evident in the liver (2.92 µequivalents/g), with lesser amounts in the salivary gland (0.31 µequivalents/g) and vertebrae (0.34 µequivalents/g). At 24 hours post dosing, greatest amounts of radioactivity were found in bone marrow (8.10 µequivalents/g), teeth (4.75 µequivalents/g) and intestinal contents. At all timepoints, liver displayed a heterogeneous speckled pattern in autoradiographs, as had been seen in a similar study in male rats.

Radioactivity was not detected in the placenta or fetuses at any of the timepoints. As was seen in male rats, radioactivity indicative of proguanil and its metabolites was limited in distribution within 24 hours of administration, without apparent penetration of the placental barrier.

| Study Title: **The rates and routes of excretion of radioactivity from male Sprague Dawley rats after oral administration of $^{14}$C-proguanil in combination with atovaquone at doses of 8mg/kg and 20mg/kg respectively** | Study No: BDRR/94/0036 | GLP compliance Yes (x) No () |
|---|---|---|
| Conducting laboratory and location: The Wellcome Foundation Beckenham, Kent, UK | Date of study finalization: 17 May 1995 | QA- Report Yes (x) No () |

Male Sprague Dawley rats (3/group) were given atovaquone (batch Q4M), 20 mg/kg and proguanil, 8 mg/kg, and $^{14}$C-proguanil ( batch CA, specific activity 113mCi/mmol) in 0.25% methyl cellulose in a single oral dose. Rats were then placed in metabolism cages

for separate quantitative collection of urine and feces. Urine was collected at intervals of 0-6, 6-24, 24-48, 48-72, 72-96, 96-120, 120-144 and 144-168 h; feces were collected every 24h up to 168 h. Most radioactivity (78.9%) was collected in the feces, 71.1% in the initial 24h. Urine contained 11.5% of the radioactivity, 9.5% emerging in the first 24h. Rat carcasses contained another 0.27% of radioactivity.

| Study Title: **Radioprofiling of plasma, urine and fecal extracts following oral administration of $^{14}$C-proguanil (8mg/kg) combined with atovaquone (20mg/kg) to the rat** | Study No: BDRR/95/0039 | GLP compliance Yes (x) No () |
|---|---|---|
| Conducting laboratory and location: GlaxoWellcome Co. Beckenham, Kent, UK | Date of study finalization: 6 March 1996 | QA- Report Yes (x) No () |

This study was conducted in conjunction with another pharmacokinetic study, BDRR/94/0035 (see above). Urine was collected from the rats at predose, 0-6 h and 6-24h and pooled within each timepoint. Feces collection was not described. Extracts of the excreta were made for analysis by — and ( — Recovery of radioactivity from the extracts showed approximately — recovery from the feces, mostly from the first 24 hours. Approximately — of radioactivity was recovered from urine. In urine, three lesser peaks in addition to proguanil were seen on — but not conclusively identified.

| Study Title: **Toxicokinetic study in pregnant rabbits given 566C89 (atovaquone) and 336U50 (proguanil HCl) in 2.5:1 ratio by gavage** | Study No: TTEP/95/0081 | GLP compliance Yes (x) No () |
|---|---|---|
| Conducting laboratory and location: Burroughs Wellcome Co. 3030 Cornwallis Rd. Research Triangle Park, NC 27709 | Date of study finalization: 11 March 1996 | QA- Report Yes (x) No () |

Female New Zealand White rabbits ( [redacted] were inseminated (day 0 of gestation) and administered atovaquone:proguanil orally daily on days 6 through 20 of gestation. Treatment groups (5 dams/group) consisted of (atovaquone:proguanil) 25:10, 50:20, 100:40, 100:0 and 0:40 mg/kg. On day 20 maternal blood was collected at 0.5, 1, 2, 4, 8, 12, 16 and 24 h following dosing. Blood was analyzed by — for drug and metabolite levels and pharmacokinetic evaluation. Rabbits were sacrificed and necropsied following the last blood collection. During the study deaths occurred in these groups: 25:10 (1=dosing accident), 50:20 (1=dosing accident) and 100:0 (1=dosing error). No treatment related gross effects were seen at terminal necropsy.

| | 25:10 mg/kg atovaquone: proguanil | 50:20 mg/kg atovaquone: proguanil | 100:40 mg/kg atovaquone: proguanil | 100:0 mg/kg atovaquone: proguanil | 0:40 mg/kg atovaquone: proguanil |
|---|---|---|---|---|---|
| Atovaquone AUC (µg/ml.h) | 51.4 | 111.8 | 170.1 | 182.6 | -- |
| Atovaquone Cmax (µg/ml) | 3.50 | 7.06 | 9.62 | 10.58 | -- |
| Proguanil AUC (µg/ml.h) | 0.584 | 1.386 | 2.868 | -- | 5.075 |
| Proguanil Cmax (µg/ml) | 0.170 | 0.306 | 0.462 | -- | 0.601 |
| Cycloguanil AUC (µg/ml.h) | 0.239 | 1.392 | 2.563 | -- | 2.041 |
| Cycloguanil Cmax (µg/ml) | 0.066 | 0.267 | 0.370 | -- | 0.284 |

Exposures to each drug increased in a linear relationship with dose. Coadministration of the drugs did not appear to affect exposure. The human equivalent doses for the range of doses used in this study range from 8.33/3.33 to 33.3/13.33 mg/kg. At the 100:40 mg/kg dose, the AUC for proguanil in pregnant rabbits (AUC= 2.87 µg/ml.h) is approximately 45% of the estimated human proguanil exposure (AUC= 6.2 µg/ml.h) during malarone treatment in humans, while cycloguanil in pregnant rabbits had an AUC ranging from 2.041 to 2.563 µg/ml.h, slightly greater than expected human exposure.

| Study Title: **The pharmacokinetics of atovaquone and proguanil in the male and female beagle dog following single oral administration of $^{14}$C-proguanil alone, or $^{14}$C-proguanil in combination with atovaquone, at dose levels of 6mg/kg proguanil and 15mg/kg atovaquone** | Study No: BDRR/94/0034 | GLP compliance Yes (x) No () |
|---|---|---|
| Conducting laboratory and location: The Wellcome Foundation. Beckenham, Kent, UK | Date of study finalization: 16 June 1995 | QA- Report Yes (x) No () |

Male and female beagle dogs ( , were administered a single oral dose of $^{14}$C-proguanil (batch CA) alone, or $^{14}$C-proguanil (batch CA) in combination with atovaquone, at dose levels of 6mg/kg proguanil and 15mg/kg atovaquone. Dogs were then placed in metabolism cages for radioactive excretion measurement (see study report BDRR/95/0038 below). Blood samples were collected at predose, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 32, 48, 56, 72, 80, 96 and 120 h following dosing. Plasma was prepared for radioactivity and analysis. Male and female pharmacokinetic values were not significantly different. Atovaquone displayed two plasma concentration peaks, at 1.5-3 h and 24-72 h, with Cmax of 5.2 and 6.5 µg/ml, respectively. The AUC was variable, ranging from 178-606 µg/ml.h. Proguanil

concentrations peaked about 1.5-3 h, with Cmax of 0.70 µg/ml and AUC from 4.2-11.6 µg/ml.h. Variable amounts of the proguanil metabolite cycloguanil appeared in the first 24h. Concentrations peaked between 2 and 6h, with Cmax of 0.10 µg/ml.

| Study Title: **The rates and routes of excretion of radioactivity from male and female beagle dogs following single oral administration of $^{14}$C-proguanil in combination with atovaquone, at dose levels of 6mg/kg proguanil and 15mg/kg atovaquone** | Study No: BDRR/95/0038 | GLP compliance Yes (x) No () |
|---|---|---|
| Conducting laboratory and location: The Wellcome Foundation. Beckenham, Kent, UK | Date of study finalization: 13 July 1995 | QA- Report Yes (x) No () |

Using urine and feces collected in the previous study (report BDRR/94/0034, above), radioactivity from $^{14}$C-proguanil alone and in combination with atovaquone was measured. In combination, — of radioactivity was recovered in the 5 day collection period. In the urine. — was recovered, —— emerging in the initial 48h. The remainder, — was collected in feces with — in the initial 48h. $^{14}$C-proguanil administered alone resulted in radioactivity recovery similar to the combination with atovaquone. Again, incomplete elimination of the radioactivity occurred after 5 days of collection. A total of — of total radioactivity was recovered, with — of total recovered in urine, — in the first 48h. Feces accounted for — of total, with — recovery after 48h.

| Study Title: **A study to investigate the effects upon hepatic drug metabolizing enzyme activity in male Sprague Dawley rats following daily oral administration of atovaquone and proguanil in the ratio 2.5:1** | Study No: BDRR/95/0050 | GLP compliance Yes (x) No () |
|---|---|---|
| Conducting laboratory and location: Burroughs Wellcome Co. 3030 Cornwallis Rd. Research Triangle Park, NC 27709 | Date of study finalization: 26 September 1995 | QA- Report Yes (x) No () |

Liver samples from the male Sprague Dawley rats of the 35-day subchronic study (see toxicology studies, below; dose groups used = 0:0, 100:40, 100:0 and 0:40 mg/kg) were examined for drug metabolizing activity following malarone treatment. Cytochrome P450 levels were measured. Testosterone hydroxylase, 7-ethoxyresorofin, O-deethylase, 7-pentoxy-resorufin O-dealkylase and 1-naphthol glucuronyltransferase activity were determined. Atovaquone alone increased total P450, proguanil alone decreased total protein. Each drug alone decreased 7-pentoxy-resorufin O-dealkylase and testosterone hydroxylase (16α and 2α) levels. The combination also slightly increased 1-naphthol glucuronyltransferase levels.

| Study Title: **Study of protein binding of proguanil and to investigate potential displacement from binding sites on plasma proteins in rat, dog and human plasma in the presence of atovaquone** | Study No: BDRR/95/0040 | GLP compliance Yes (x) No () |
|---|---|---|
| Conducting laboratory and location: Burroughs Wellcome Co. 3030 Cornwallis Rd. Research Triangle Park, NC 27709 | Date of study finalization: 10 November 1995 | QA- Report Yes (x) No () |

Fresh blood from rats, dogs, and humans was collected and separated into plasma. $^{14}C$-proguanil (batch CB) and $^{14}C$-atovaquone (batch CV) as well as non-labeled proguanil (batch XA) and atovaquone (batch Q4M) were inoculated into the samples, incubated, and plasma proteins separated by ultrafiltration and analyzed for radioactive content. Drug concentrations ranged from 0.01-10 µg/ml. Atovaquone exhibited high affinity for plasma protein (>99%) in each of the species. Proguanil plasma protein binding affinity varied, from about 65% in the rabbit, 81% in the dog and rat, and 75% in human plasma. Atovaquone slightly displaced proguanil in the rat and dog, decreasing relative binding to 77% and 76 %, respectively. Proguanil did not affect binding of atovaquone in any of the samples tested.

| Study Title: **A study of the metabolism in vitro of proguanil using hepatic microsomes prepared from rat, rabbit, dog, monkey and human tissue** | Study No: BDRR/95/0047 | GLP compliance Yes (x) No () |
|---|---|---|
| Conducting laboratory and location: GlaxoWellcome Co. Beckenham, Kent, UK | Date of study finalization: 27 November 1995 | QA- Report Yes (x) No () |

Hepatic microsomal preparations from rat, rabbit, dog, monkey, and man were prepared and incubated with proguanil for 30 and 60 min. Preparations were analyzed by [redacted] for metabolite production and identification. 4-chlorophenyl biguanide and cycloguanil, the major metabolites of proguanil, were produced in significantly greater quantity in the rabbit than other species, of which the monkey produced more than dog, rat and human..

| Study Title: **A study to investigate the in vitro metabolism of proguanil using hepatocytes isolated from rat, rabbit, dog, cynomologus monkey and human** | Study No: BDRR/95/0048 | GLP compliance Yes (x) No () |
|---|---|---|
| Conducting laboratory and location: GlaxoWellcome Co. Beckenham, Kent, UK | Date of study finalization: 4 August 1995 | QA- Report Yes (x) No () |

Hepatocytes from rat, rabbit, dog, monkey, and man were prepared and incubated with proguanil for 30 and 60 min. Preparations were analyzed by — for metabolite production and identification. 4-chlorophenyl biguanide and cycloguanil were produced by all animal species, with greater amounts of the biguanide relative to cycloguanil. In the human hepatocytes, greater amounts of cycloguanil were produced compared to biguanide. The relative extent of metabolism was similar to that seen above in the microsomal preparations, with the rabbit preparation displaying significantly greater activity than the other species tested.

| Study Title: **A study to investigate the potential for the glucuronidation of atovaquone by hepatic enzymes preparations from animals and man** | Study No: BDNG/94/0005 | GLP compliance Yes (x) No () |
|---|---|---|
| Conducting laboratory and location: The Wellcome Foundation. Beckenham, Kent, UK | Date of study finalization: 27 November 1995 | QA- Report Yes (x) No () |

Hepatic microsomal preparations from rat, rabbit, dog, monkey, and man were prepared and incubated with atovaquone and UDP-glucuronic acid. Preparations were analyzed by — Atovaquone was not metabolized to a glucuronide conjugate by microsomes from any of the species tested.

**Pharmacokinetic Summary**

The studies presented were designed to determine possible pharmacokinetic interaction between atovaquone and proguanil, including protein binding, plasma levels, in vitro hepatic preparations for metabolism, as well as the previously uncharacterized pharmacokinetic properties of proguanil alone. In rats, dogs, and rabbits atovaquone has limited bioavailability, with limited systemic exposure, extensive plasma protein binding and fecal excretion as the unchanged ~~the unchanged~~ drug. Proguanil is metabolized to its major active metabolite, cycloguanil and the inactive metabolite 4-chlorophenyl-biguanide (biguanide) in all species examined. Unfortunately, recovery of labeled cycloguanil was not attempted in these studies to follow the disposition of proguanil.

Radiolabeled proguanil studies in rats showed proguanil rapidly disappeared. In pregnant female rats, proguanil was quantifiable in plasma only at a dose of 20 mg/kg. Whole body radiography of male rats showed radiolabeled proguanil distributing mainly to the liver within 24h. A similar study in pregnant female rats showed radiolabeled proguanil mainly in maternal liver and not in the fetuses. Excretion of label appeared predominantly in feces in the initial 24h following administration. Atovaquone and proguanil did not significantly affect coexposure.

Pregnant female rabbits had limited exposure to proguanil, providing approximately 45% of expected human treatment exposure. This is an interesting finding as the in vitro hepatic findings (see below) rabbit hepatic preparations (cells, enzymes and microsomes) consistently metabolized proguanil to metabolites more readily than preparations from

other species. Cycloguanil exposure at a dose of 40 mg/kg proguanil was 1.3 to 1.6 fold greater than that seen at an expected human dose. Metabolism to biguanil may account for a larger portion of total metabolism in rabbits than in humans, resulting in underestimation of maternal exposure.

In dogs, urine was the main route of excretion of labeled proguanil. Most radiolabeled proguanil was excreted in the initial 48h. Significant amounts of radioactivity (22%) remained unexcreted five days after dosing. It appears that a single dose of proguanil may reside for prolonged time periods in dogs. In view of the toxicity findings in the six-month dog toxicology study, more detailed pharmacokinetics to assess proguanil exposure (including cycloguanil) and radiolabeled distribution studies would have enabled correlation between exposure, organ/tissue sensitivity and toxicity.

***In vitro***

Plasma protein binding for atovaquone is >99% in all species tested while plasma protein binding for proguanil ranges from about 65 to 80 %, without either significantly affecting the binding of the other. In all hepatic preparations (hepatic enzymes, hepatocytes and microsomes), those from rabbits metabolize proguanil *in vitro* significantly greater than preparations from other species in terms of total metabolite production. This must be taken into account when comparing rabbit proguanil exposure to possible human exposure. As both atovaquone and proguanil each have limited oral absorption in animals, results of the toxicology and reproductive toxicology studies need to be interpreted with the pharmacokinetic data in mind when extrapolating to clinical exposure.

## TOXICOLOGY

See also Histopathology inventory for toxicology studies, p25

A one month oral toxicity study in Beagle dogs given 566C80 (atovaquone) and proguanil HCl in 2.5:1 ratio. See IND ——— attached

A six month oral toxicity study in Beagle dogs given 566C80 (atovaquone) and proguanil HCl in 2.5:1 ratio

A one month oral toxicity study in Han Wistar rats given 566C80 (atovaquone) and proguanil HCl in 2.5:1 ratio. See IND ——— attached

A six month oral toxicity study in Han Wistar rats given 566C80 (atovaquone) and proguanil HCl in 2.5:1 ratio

## GENETIC TOXICOLOGY

Evaluation of proguanil hydrochloride (336U50) for mutagenicity using the Ames Salmonella/microsome plate incorporation test and the pre-incubation modification

L5178/tk+/- mouse lymphoma mutagenesis study with 336U50

A micronucleus assay in mice with [redacted] hydrochloride

An oral carcinogenicity study with 566C80 in [redacted] CD-1 mice
IND [redacted] (attached)

An oral carcinogenicity study with 566C80 in [redacted] CD rats
IND [redacted] (attached)

## REPRODUCTIVE TOXICOLOGY

Teratology study in rats given 566C80 (atovaquone) and 336U50 (proguanil HCl) in 2.5:1 ratio by gavage. See IND [redacted] attached

Teratology study in rabbits given 566C80 (atovaquone) and 336U50 (proguanil HCl) in 2.5:1 ratio by gavage

## TOXICOLOGY REPORTS:

| Study Title: **A six month oral toxicity study in Beagle dogs given 566C80 (atovaquone) and proguanil HCl in 2.5:1 ratio** | Study No:D40208 | GLP compliance Yes (x) No () |
|---|---|---|
| Conducting laboratory and location: [redacted] | Date of study finalization: 19 Nov 1997 | QA- Report Yes (x) No () |

Beagle dogs ( [redacted] 8-9 months) were place in dose groups (4/sex/group) receiving (atovaquone:proguanil) 0:0, 10:4, 20:8, 30:12, 30:0 and 0:12 mg/kg by oral gavage daily for six months. Recovery dogs (2/sex/group) received (atovaquone:proguanil) 0:0, 30:12, 30:0, 0:12 mg/kg over the same period and recovered for an additional four weeks.

| | |
|---|---|
| Drug, lot# | (Atovaquone: Proguanil) 344001A, P684R |
| Formulation/vehicle | Powder in gelatin capsule |

Observations and times:

| | |
|---|---|
| Clinical signs | Twice daily |
| Body weights | Weekly |
| Food consumption | Weekly |
| Ophthalmoscopy | Prior to start of dosing, during weeks 13 and 26 |
| EKG | Pretreatment, week 13 and 26 |
| Hematology | Weeks 4, 13, 26 |
| Clinical chemistry | Weeks 4, 13, 26 |
| Urinalysis | Weeks 4, 13, 26 |
| Organ weights | Terminal necropsy |
| Gross pathology | Terminal necropsy |
| Organs weighed | Terminal necropsy |
| Histopathology | Terminal necropsy |
| Toxicokinetics | Blood collected day 1 and week 26 , predose, 1, 2, 4, 6, 8, 12, 16 and 24h post dose |

Deaths

| Group Atovaquone :proguanil, mg/kg | ♂ | ♀ |
|---|---|---|
| 0:0 | | |
| 10:4 | | |
| 20:8 | | |
| 50:20[a] → 40:16[b] →30:12[c] | 2, Week 22, 23 | 1, week 7 |
| 50:0[a] → 40:0[b] → 30:0[c] | | |
| 0:20[a] → 0:16[b] → 0:12[c] | 3, week 5, 6, 21 | 1, week 27 |

[a] weeks 1-4
[b] weeks 5-8
[c] weeks 9-26

During the study, 5 30:12 mg/kg dose group dogs (week 7, 22, 23) and four 0:12 mg/kg dose group dogs (week 5, 5, 21, 27) were found dead or euthanized between days 40 and 170. In the dead in the 30:12 and 0:12 mg/kg dose groups, findings at death included bone marrow hypocellularity, gastritis/enteritis, and lymphoid atrophy. Decreased body weight (weight loss) and food consumption were observed in dogs in the 30:12 and 0:12 mg/kg dose groups during the first month of dosing. After the first dose decrease at week 5, body weights and food consumption reportedly increased to levels comparable to those of the vehicle control group. These values for males in the 30:12 mg/kg dose group, however, remained below control values. Emesis and salivation were reported in groups 4 and 6 through the study. Soft and liquid feces were seen in the 20:8, 30:12, and 0:12 mg/kg dose groups. Severity was related to proguanil dose. At week 26, electrocardiography was performed showing two 30:12 mg/kg dose group females with

an inverted ventricular extrasystole. One of these females and a 30:12 mg/kg dose group female had notched P waves and one group 6 female had sinus tachycardia. At week 4, a slight decrease in RBC count was seen in the 20:8, 30:12, and 0:12 mg/kg dose groups. Recovery was seen following reduction of doses.

At necropsy, gross findings were mainly seen in the 30:12 and 0:12 mg/kg dose groups and included emaciation, dark/raised areas, thickening or mass in right atrium, dark foci/areas in the gall bladder, and depressed areas in the duodenum and colon. Histopathology findings included fibrovascular proliferation of the heart in the 30:12 and 0:12 mg/kg dose group dogs at the main necropsy and in the recovery group; bile duct hyperplasia and gall bladder lesions (mucosal atrophy with or without inflammation and pigment accumulation) in all groups receiving proguanil; slight to moderate pyelitis in one surviving female from the 0:12 mg/kg dose group; mild pyelonephritis in one female, 0:12 mg/kg dose group, severe pyelonephritis in one surviving male in the 0:12 mg/kg dose group, severe interstitial nephritis in a surviving female in the 30:12 mg/kg dose group; thymic and lymphatic atrophy in dogs in the 30:12 and 0:12 mg/kg dose groups, with the most severe cases in those dogs dying during study (signs not seen in recovery dogs); interstitial pneumonia in all groups but most severe in the 20:8, 30:12, and 0:12 mg/kg dose groups; slight multifocal necrosis with brain hemorrhage, one 0:12 mg/kg dose group female and bone marrow hypocellularity and gastritis/enteritis in the dogs dying during study. Bile duct hyperplasia of the liver, gall bladder mucosal atrophy and epithelial hyperplasia of the lung were seen in all proguanil-treated groups, including the low dose, 10:4 mg/kg group.

Following the one month recovery (0:0, 30:12, 30:0 and 0:12), interstitial pneumonia was present in all treated groups, as well as lesions seen in the heart, liver, and gall bladder A NOAEL was not achieved in this study as bile duct hyperplasia of the liver, gall bladder mucosal atrophy and interstitial pneumonia with epithelial hyperplasia was seen in the lowest dose group (10:4 mg/kg). The combination of thymic and lymphoid atrophy with interstitial pneumonia, which the sponsor characterized by "...the incidence and severity of typical background pathology in the lungs of dogs, both control and treated, in this 6 month study were unusually high" raises concerns of possible immunosuppressive activity by proguanil. DHFR inhibitors are known to cause immunosuppression. Human equivalent doses for the range of doses used in this study range (10:4 to 30:12 mg/kg in dogs) range from 5:2 to 15:6 mg/kg which compares to the treatment dose of 20:8 mg/kg and the prophylaxis dose of 5:2 mg/kg. Pharmacokinetic parameters from the plasma samples yielded a mean AUC (males and females) of approximately 8.8 µg.h/ml from the 50:20 mg/kg group and 6.5 µg.h/ml from the 0:20 mg/kg group after 26 weeks. This compares to an AUC for the 8 mg/kg proguanil treatment dose in humans of about 6.2 µg.h/ml, or 1 to 1.4 times expected human exposure during treatment. Estimates based on a human prophylaxis dose exposure (100 mg, 2 mg/kg; pharmacokinetic studies were not performed at this dose in humans, therefore are based on half of the AUC based on a 200 mg dose and linear pharmacokinetics) places the dog exposure at 5 to 7 times human exposure. Cycloguanil AUC in dogs receiving 40 mg/kg proguanil ranged from 1.5 to 2.0 µg.h/ml, or similar to the exposure observed in humans (1.0 µg.h/ml from 200 mg proguanil dose).

| Study Title: **A six month oral toxicity study in Han Wistar rats given 566C80 (atovaquone) and proguanil HCl in 2.5:1 ratio** | Study No:R40209 | GLP compliance Yes (x) No () |
|---|---|---|
| Conducting laboratory and location: [redacted] | Date of study finalization: 14 Nov 1997 | QA- Report Yes (x) No () |

Han/Wistar rats ( [redacted] 8 weeks old) were placed in dose groups (20/sex/group) receiving (atovaquone:proguanil) 0:0, 10:4, 20:8, 50:20, 50:0, 0:20 mg/kg by daily oral gavage for six months. Recovery groups (12/sex /group) received (atovaquone:proguanil) 0:0, 50:20, 50:0 and 0:20 mg/kg over the same period and recovered for an additional four weeks.

| | |
|---|---|
| Drug lot# | 344001A atovaquone; P684R, proguanil |
| Formulation/vehicle | [redacted] |

Observations and times:

| | |
|---|---|
| Clinical signs | twice daily |
| Body weights | Weekly |
| Food consumption | Weekly |
| Ophthalmoscopy | Prior to start of dosing, during weeks 13 and 25 |
| EKG | |
| Hematology | Weeks 4, 13, 27 |
| Clinical chemistry | Weeks 4, 13, 27 |
| Urinalysis | Weeks 4, 13, 26 |
| Organ weights | Terminal necropsy |
| Gross pathology | Terminal necropsy |
| Organs weighed | Terminal necropsy |
| Histopathology | Terminal necropsy |

Results: deaths

| Group Atovaquone :proguanil, mg/kg | ♂ | ♀ |
|---|---|---|
| control | 1, day 178 | |
| 10:4 | 1, day 39 | 1: day 154 |
| 20:8 | | |
| 50:20 | | 5: day 76, 77, 86, 140, 162, |
| 50:0 | 1, day 101 | |
| 0:20 | | 3: day 14, 110, 144, |

During the study, no clinical signs were seen. At necropsy, group mean body weights were decreased in males in the 20:8, 50:20 and 0:20 mg/kg groups beginning at week 11 and through recovery. Female body weights were unaffected and food consumption was unaffected in both sexes. Organ weight changes in the 20:8, 50:20, and 0:20 mg/kg groups were seen including increases in the relative organ weights of brain, heart, liver, lung, kidney, and testis. Microscopic findings included increased incidence of tubular basophilia in 50:20 and 0:20 mg/kg groups and dose related increases in mucosal hyperplasia of cecum in all proguanil treated groups. Following recovery, slight basophilia remained in the 50:20 mg/kg group.

A NOAEL was not achieved in this study as cecal hyperplasia was seen in the lowest dose group (10:4 mg/kg). Rats receiving atovaquone alone had no adverse effects. Human equivalent doses for the range of doses used in this study range (10:4 to 50:20 mg/kg in rats) range from 1.66:0.66 to 8.33:3.33 mg/kg.

## SUMMARY OF ANIMAL TOXICOLOGY STUDIES

| | 1month rat | 1month dog | 6 month rat | 6 month dog |
|---|---|---|---|---|
| NOAEL, mg/kg | 100:40 | 50:20 | 20:8 | < 10:4 |
| NOAEL HED (for proguanil) | 16.6: 6.6 | 25:10 | 3.3:1.3 | < 5:2 |
| AUC µg.h/ml | | | 680:0.22 | 86:8.8 |
| Proguanil toxicity seen at dose x | | | 50:20 | ≥10:4 |
| Margin versus human dose | | | 0.035X | 1.4X |

Human doses 5:2 mg/kg=prophylaxis 20:8 mg/kg=treatment,
proguanil AUC =6.2 µg.h/ml HED=human equivalent dose

## GENETIC TOXICOLOGY

| Study Title: **Evaluation of proguanil hydrochloride (336U50) for mutagenicity using the Ames Salmonella/microsome plate incorporation test and the pre-incubation modification** | Study No: BDRE/94/0396 | GLP compliance Yes (x) No () |
|---|---|---|
| Conducting laboratory and location: The Wellcome Foundation Beckenham, UK | Date of study finalization: July 7, 1995 | QA- Report Yes (x) No () |

Proguanil was evaluated in the Ames test using five strains of *Salmonella typhimurium* (TA1535, TA1537, TA98, TA100 and TA102) in order to detect mutations caused by either basepair frameshift or substitution. Proguanil (batch XC) was prepared in [redacted] providing concentrations of 2.0, 0.632, 0.2, 0.06332 and 0.02 mg/ml, or 200, 63.2, 20, 6.32 and 2 µg/plate, based on preliminary toxicity tests with strain TA100.

Positive controls included sodium nitrate, 2-aminoanthracene, 9-aminoacridine, azaserine, mitamycin C, and 4-nitro-O-phenylene-diamine. All positive controls were effective, with or without appropriate S9 induction. Rat liver S9 preparations were induced using phenobarbitone and β-napthoflavone. Cytotoxicity was observed at the 200 µg/plate concentration with the TA102 strain. Proguanil did not induce dose- or metabolic activation-related increases in revertant counts in any of the *S. typhinurium* test strains.

| Study Title: **L5178/tk+/- mouse lymphoma mutagenesis study with 336U50** | Study No: TTEP/95/0023 | GLP compliance Yes (x) No () |
|---|---|---|
| Conducting laboratory and location: Burroughs Wellcome Co. Research Triangle Park, NC | Date of study finalization: October 27, 1995 | GLP compliance Yes (x) No () |

Mouse lymphoma L5178Y TK+/- cells were used in this study to determine the potential of Proguanil to induce mutations or chromosomal damage at the thymidine kinase locus. Test compound (batch not given) and positive controls (with S-9, methyl methanesulfonate; without S-9, 3-methylcholanthrene) were [redacted] Experiments were conducted in the presence and absence of S-9 preparations from aroclor-induced rat livers. Two experiments were performed, the first to a determine drug concentration range, and the second, a formal assay. The initial experiment used concentrations of proguanil ranging from 20 to 5000 µg/ml. Excessive cytotoxicity resulted at concentrations above 20 µg/ml. In the formal assay, proguanil concentrations of 2.5 to 37.5 µg/ml were used without metabolic activation; clones were prepared to determine mutation frequency from cultures grown between 10 and 27.5 µg/ml. Concentrations from 5 to 75 µg/ml were used with metabolic activation; clones were prepared to determine mutation frequency from cultures grown between 10 and 45µg/ml. There was no significant increase in the mutant frequency of treated cultures relative to solvent controls, with or without metabolic activation.

| Study Title: A **micronucleus assay in mice with 336U50 hydrochloride** | Study No: TTEP/95/0052 | GLP compliance Yes (x) No () |
|---|---|---|
| Conducting laboratory and location: Burroughs Wellcome Co. Research Triangle Park, NC | Date of study finalization: November 14, 1995 | QA- Report Yes (x) No () |

Male and female CD-1 mice, 5/sex/timepoint, six weeks old ( [redacted] ) were administered Proguanil (batch not listed) or cyclophosphamide (positive control) in the below schedule.

| Dose (mg/kg/day) | Sacrifice after final dose (h) | Dose schedule | Dead |
|---|---|---|---|
| 0 | 24 | Uid, 3x | |
| 0 | 24 | " | |
| 0 | 24 | " | |
| 15 | 24 | " | |
| 30 | 24 | " | |
| 45 | 24 | " | 1 male, 5 females |
| 60 | 24 | " | 2 males, 4 females |
| 75 | 24 | " | 4 males, 4 females |
| Cyclophosphamide, 40 | 24 | Uid, 1x | |
| Cyclophosphamide, 60 | 24 | Uid, 1x | |
| 45 (plasma sample) | 4 | Uid, 3x | See above |
| 60 (plasma sample) | 4 | Uid, 3x | See above |

Bone marrow from the femur was processed immediately following sacrifice and examined for micronuclear polychromatic erythrocytes. Positive controls were effective for inducing significantly increased counts of micronucleated polychromatic erythrocytes. A statistically significant increase in the percent polychromatic erythrocytes in males treated with 45 mg/kg ($p=0.046$) and in the number of micronucleated polychromatic erythrocytes for male mice treated with 15 mg/kg ($p=0.032$). There were no dose-related effects seen in this assay.

Plasma drug concentrations in the 45 mg/kg group were measured 4 hours after the last dose and ranged from [redacted] (mean 0.63 µg/ml males, 0.77µg/ml females).

## REPRODUCTIVE TOXICOLOGY

| Study Title: **Teratology study in rabbits given [redacted] (atovaquone) and [redacted] proguanil HCl) in 2.5:1 ratio by gavage** | Study No: TTEP/95/0082 | GLP compliance Yes (x) No () |
|---|---|---|
| Conducting laboratory and location: Burroughs Wellcome Co. 3030 Cornwallis Rd. Research Triangle Park, NC 27709 | Date of study finalization: 6 May 1996 | QA- Report Yes (x) No () |

Female New Zealand White rabbits ( [redacted] , 6-8 months old) were given i.v. gonadotropin for induction of ovulation and artificially inseminated from semen of proven bucks. Day 0 of gestation was designated by the day of insemination. Does were placed in dose groups (25/group) and given atovaquone:proguanil doses of 0:0, 25:10, 50:20, 100:40, 100:0 and 0:40 mg/kg by daily oral gavage on gestation days 6-20. For dose selection, GlaxoWellcome cites a previous dose range finding study which was not provided.

Observations and times:

| | |
|---|---|
| Clinical signs | Daily (mortality 2x daily) |
| Body weights | Gestation days 0, 6, 9, 12, 15, 18, 20, 21 and prior to sacrifice on day 29 |
| Food consumption | Gestation days 0-6, 6-10, 10-15, 15-20, 20-24, 24-29 |
| Organ weights | At laparotomy (gestation day 29) |
| Gross pathology | At laparotomy (gestation day 29) |
| Organs weighed | At laparotomy (gestation day 29) |

Does were sacrificed on day 29 and gravid uterine weights were measured. Corpora lutea, implantation sites, live fetuses, dead fetuses, and early and late resorptions were enumerated. Fetuses were weighed, measured (crown rump distance) and examined for external malformations. Fetuses were sexed, dissected and examined for visceral abnormalities (Staple's technique). Fetal eyes were removed to determine size, shape, opacity or gross abnormalities. All fetuses were cleared and stained with Alizarin Red S and examined for skeletal defects.

maternal deaths

| Group Atovaquone :proguanil, mg/kg | Aborted and sacrificed | died | Dosing accident | Undetermined |
|---|---|---|---|---|
| Control | | | | |
| 25:10 | | | | |
| 50:20 | | 4 | 2 | 2 |
| 100:40 | 1 | 10 | 3 | 7[a] |
| 100:0 | 1 | 1 | | 1 |
| 0:40 | 0 | 2 | | 2 |

[a] includes autolyzed dead

Clinical signs during study: Decreased activity, prostration, loss of righting reflex, ataxia, and red material around the anus were seen in does of the 100:40 mg/kg group.
Body weight /food consumption changes: body weight of the 100:40 mg/kg does was significantly decreased on gestation days 12, 15, 18, 20 and 21. This group also had significantly reduced weight gain on gestation days 6-9, 12-15 and 6-20. The 100:0 mg/kg group also had reduced weight gain on days 12-15. Maternal food consumption was significantly decreased in the 100:40 mg/kg group on gestation days 6-10, 10-15, 15-20 and 20-24 as well as in the 0:40 mg/kg group on days 6-10. Erosion of the gastric mucosa was also seen in the 100:40 mg/kg group.

Malarone effects on pregnancy

| Group Atovaquone :proguanil, mg/kg | Females on study | Died | Aborted | Gravid | % pregnant | Litters |
|---|---|---|---|---|---|---|
| Control | 25 | | | 21 | 84.0 | 21 |
| 25:10 | 25 | | | 20 | 80.0 | 20 |
| 50:20 | 25 | 4 | | 17 | 68.0 | 14 |
| 100:40 | 23 | 8 | 1 | 16 | 69.6 | 8 |
| 100:0 | 25 | 1 | 1 | 19 | 76.0 | 16 |
| 0:40 | 25 | 2 | | 19 | 76.0 | 17 |

Malarone effects on fetuses

| Group Atovaquone :proguanil, mg/kg | Live fetuses M | F | total | total/litter | Dead fetuses | Resorptions Early | late | Malformations Total | external | soft tissue | skeletal |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 58 | 51 | 109 | 5.19 | 1 | 8 | 7 | | | | |
| 25:10 | 60 | 56 | 116 | 5.80 | 1 | 11 | 2 | 2 | | 1 | 1 |
| 50:20 | 46 | 36 | 82 | 5.86 | | 3 | 1 | 3 | 2 | | 1 |
| 100:40 | 20 | 30 | 50 | 6.25 | 2 | 6 | 1 | | | | |
| 100:0 | 46 | 56 | 102 | 6.38 | | 6 | 3 | 3 | | 2 | 1 |
| 0:40 | 59 | 40 | 99 | 5.82 | | 8 | 1 | 1 | | | 1 |

At the highest combined dose, there appears to be a decrease in the number gravid does as well as the number of litters relative to the other groups. This coincides with the maternal toxicity observed at this dose, including death and decreased body weight gain and food consumption. There were no dose-related increases in the number of malformations in the drug-treated groups. Interestingly, all malformations were observed only in fetuses from drug-treated dams, but not from the dams receiving the highest combined doses of atovaquone and proguanil. The NOAEL for this study appears to be 25:10 mg/kg in pregnant rabbits, equivalent to a dose of 8:3.3 mg/kg in humans. See the study "Toxicokinetic study in pregnant rabbits given 566C89 (atovaquone) and 336U50 (proguanil HCl) in 2.5:1 ratio by gavage" below for pharmacokinetic information.

**Toxicology Summary**

In all toxicity studies, proguanil was shown to produce toxicities in rats and dogs when given alone or in combination with atovaquone. Atovaquone alone given at the highest dose did not produce any toxic effects. In previous atovaquone toxicity studies, drug exposure was limited due to low oral availability. Toxicity was not seen in rats or dogs in one- and six-month studies. Exposure was limited relative to expected human exposure. In combination with proguanil, atovaquone exposure in rats were similar to human treatment exposure while dogs were exposed to approximately 0.1 to 0.2 times the expected human exposure. Atovaquone doses were limited by proguanil toxicity in dogs. Proguanil demonstrated a variety of toxicities in the rat and especially dog studies. In rats, cecal epithelial loss and erosions of the gastric mucosa were the major findings at one month in rats receiving 40 mg/kg proguanil. At six months, the gastrointestinal findings were again present but reversible. Dogs at one month receiving 40 mg/kg proguanil also displayed gastrointestinal erosions.

The paucity of pharmacokinetic studies with proguanil in dogs, especially tissue distribution and potential accumulation, hinders interpretation of the six-month dog toxicity study. The mechanism of proguanil toxicity are unclear. Whether the parent compund or metabolites cycloguanil and biguanil produce toxicity is not known. Differences between dog and human metabolite production may also account for differing sensitivity to proguanil. It is not known whether the dog has greater sensitivity to the effects of DHFR inhibitors than other species. Also, the DHFR inhibitory activity of proguanil relative to that of known inhibitors is unclear. Efforts to obtain such information from the sponsor did not provide clarification.

The toxicities seen in the six-month study occur at exposures not significantly greater than expected human exposure in malaria treatment. Unless the dog is especially sensitive to proguanil, this raises concern for human exposure at equivalent levels. Longer term exposure to proguanil might be expected to produce more severe toxicity in dogs, especially based on the antiproliferative effects seen in the thymus and lymphatic system which may manifest as immunosuppression. These effects were seen in the one- and six month study in dogs receiving 40 mg/kg proguanil, with the more severe examples occurring in the dogs which died during the study. Fibrovascular proliferation of the heart occurred only in the six-month study at 40 mg/kg. Liver, gall bladder and lung histopathologic changes not seen in the one-month study appear at the lowest proguanil dose in the six-month study. These findings may indicate a threshold for toxicity which decreases with exposure duration. While the multiple of exposure is greater for malaria prophylaxis in humans, the consequences of prolonged exposure to proguanil in prophylaxis are unclear.

**Reproductive Toxicology Summary**

Atovaquone did not have teratogenic effects in rats at plasma exposure up to 6.5 that expected in malaria treatment in humans. In rabbits, maternal toxicity limited studies to doses providing exposure 0.6 to 1.3 times that expected in malaria treatment in humans. See NDA 20-259 for review. Proguanil did not have teratogenic effects in rats at exposures about 0.1 times that of expected human exposure in malaria treatment. It is unclear as to why higher doses were not employed as maternal toxicity was not achieved in this study. Proguanil did not have teratogenic effects in rabbits at exposures up to 0.42 to 0.82 times that expected in malaria treatment in humans. Maternal toxicity limited the doses which could be employed.

Designation as a pregnancy category C drug would be appropriate as segment I and III studies for proguanil have not been submitted, the absence of well-controlled studies in pregnant humans and the benefit from the use of malarone in pregnant women may be acceptable despite possible risk.

It is not known if atovaquone is excreted into the milk in humans; in rats 30 % of the concurrent plasma concentration were found. Proguanil is known to be excreted into human milk.

**Genetic Toxicology Summary**
Proguanil and atovaquone were each not genotoxic in any of the three assays performed, with or without metabolic activation: the Ames' mutagenicity assay, the mouse lymphoma assay or the mouse micronucleus assay. Atovaquone was also negative in the cultured human lymphocyte assay. The combination was not tested for genotoxicity. Atovaquone was not carcinogenic in a 24-month rat study. In a 24-month mouse study, dose-related increases in hepatocellular adenoma and hepatocellular carcinoma occurred. Carcinogenicity bioassays with proguanil have not been completed but will be performed by GlaxoWellcome as phase IV commitments.

**CONCLUSIONS**
NDA 20-259 is approvable with respect to pharmacology/toxicology. Despite reservations regarding toxicity seen in the six-month dog study due to proguanil, clinical practice abroad has not demonstrated such toxicities.

Please refer to the labeling changes below.

**REQUESTS**
The following revised text should be considered for incorporation into the sponsor's label. These revisions are based upon labeling specified in 21CFR 201.57. Appropriate text is proposed. Word changes are underlined

1. Carcinogenesis and mutagenesis:
Carcinogenicity bioassays with proguanil have not been completed.

Proguanil was not genotoxic in *in vitro* or *in vivo* studies.

Genotoxicity studies have not been performed with atovaquone in combination with proguanil.

2. Pregnancy:
An appropriate comparison of animal doses to human doses should be made for proguanil.

3. Animal Pharmacology and Toxicology:
The reversibility of the toxicities observed in the six-month dog study should be provided.

/S/
S.C. Kunder, Ph.D.
Reviewing Pharmacologist

**concurrences:**
HFD-590/ADir/RAlbrecht 1/51
HFD-590/SPharm/KHastings 1/51
Steven C. Kunder/Pharm/

**disk:**
HFD-590/KHastings
**cc:**
HFD-590 (original)
HFD-590 Division file
HFD-340
HFD-590/MDempsey
HFD-590/AMeterhoff
HFD-590/JSmith
HFD-590/SBala
HFD-345/

## Histopathology Inventory for Toxicology Studies

| Study /Species | 1month rat | 6 month rat | 1 month dog | 6 month dog |
|---|---|---|---|---|
| Adrenals | X,w | X,w | X,w | X,w |
| Aorta | X | X | x | x |
| Bone Marrow smear | X | X | x | x |
| Bone (femur) | X | X | x | x |
| Brain | X,w | X,w | X,w | X,w |
| Cecum | X | X | x | x |
| Cervix | X,w | X,w | X | X |
| Colon | X | X | x | x |
| Duodenum | X | X | x | x |
| Epididymis | x | x | X,w | X,w |
| Esophagus | x | x | x | x |
| Eye | x | x | x | x |
| Fallopian tube | | | | |
| Gall bladder | | | x | x |
| Gross lesions | x | x | x | x |
| Harderian gland | x | x | | |
| Heart | X,w | X,w | X,w | X,w |
| Hyphophysis | | | | |
| Ileum | x | x | x | x |
| Injection site | | | | |
| Jejunum | x | x | x | x |
| Kidneys | X,w | X,w | X,w | X,w |
| Lachrymal gland | | | | |
| Larynx | | | | |
| Liver | X,w | X,w | X,w | X,w |
| Lungs | X,w | X,w | X,w | X,w |
| Lymph nodes, cervical | | | | |
| Lymph nodes mandibular | X | X | | |
| Lymph nodes, mesenteric | X | X | x | x |
| Mammary Gland | X | X | x | x |
| Nasal cavity | | | | |
| Optic nerves | X | X | x | x |
| Ovaries | X,w | X,w | X,w | X,w |
| Pancreas | X | X | x | x |
| Parathyroid | W | W | w | w |
| Peripheral nerve | | | | |
| Pharynx | | | | |
| Pituitary | X,w | X,w | X,w | X,w |
| Prostate | X,w | X,w | X,w | X,w |
| Rectum | X | X | x | x |
| Salivary gland | X | X | x | x |
| Sciatic nerve | | | x | x |
| Seminal vesicles | X | X | | |
| Skeletal muscle | X | X | x | x |
| Skin | X | X | x | x |
| Spinal cord | X | X | x | x |
| Spleen | X,w | X,w | X,w | X,w |
| Sternum | X | X | x | x |
| Stomach | X | X | x | x |
| Testes | X,w | X,w | x | x |
| Thymus | X,w | X,w | X,w | X,w |
| Thyroid | X,w | X,w | X,w | X,w |
| Tongue | X | X | x | x |
| Trachea | X | X | x | x |
| Urinary bladder | X | X | x | x |
| Uterus | X,w | X,w | X,w | X,w |
| Vagina | x | x | x | x |
| Zymbal gland | | | | |

X=histopathology examination
w= organ weight obtained

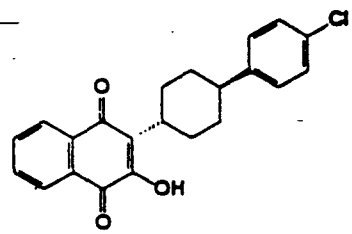
**PHARMACOLOGIST'S REVIEW**

**IND #:** [redacted] (N-085)

**DATE SUBMITTED:** Aug. 12, 1996
**DATE ASSIGNED:** Aug. 12, 1996
**DATE REVIEW COMPLETED:** March 26, 1997
**HFD-530**

**SPONSOR:** Glaxo Wellcome Inc.
Five Moore Drive
Research Triangle Park, NC 27709

**DRUG:** Atovaquone (Mepron®; 566C80)
**CHEMICAL NAME:** *trans*-2-[4-(4-chlorophenyl)cyclohexyl]-3-hydroxy-1,4-naphthalenedione
**CAS REGISTRY #:** 95233-18-4
**CHEMICAL STRUCTURE:**




**RELATED DOCUMENT:** NDA 20-500

**INDICATION:** Treatment of *Pneumocystis carinii* pneumonia (PCP)

**BACKGROUND**

Atovaquone is currently marketed for treatment of HIV-infected patients with PCP. This submission contains the report of a carcinogenicity bioassay in rats that was conducted as part of Phase IV commitments.

**NONCLINICAL TOXICOLOGY**

*An oral carcinogenicity study with 566C80 in* [redacted] *CD rats*
*Study # RD1996/00241; study dated 6-27-96*

[75/sex/dose (with 2 dietary control groups); 750 total; dietary administration for 22 months (females; 670 - 674 consecutive days) or 24 months (males; 740 - 744 consecutive days); doses used: 20, 100, 500 mg/kg/day; performed by the sponsor; drug lot # 566C80WS (reference # 89/0013-003-4), 566C80WAA (reference # 88/0001-158); dietary controls; GLP study]

The sponsor determined drug concentrations in diet admixtures after preparation of each batch. The animals were ~ 47 days of age at the start of the study.

The following survival rates (expressed as percentages) were observed at the end of the study (with doses given in parentheses): (males), 57 (0), 41 (0), 41 (20), 43 (100), 53 (500); (females), 52 (0), 44 (0), 36 (20), 67 (100), 64 (500). There was no effect of treatment on survival. The only treatment-related clinical sign observed on study was pink pelage due to the color of the drug. There was a slight decrease in mean body weight gains in high-dose group males during the second year of the study. No other body weight effects were observed. Food consumption averaged 8% and 5% greater in males and females, respectively, in the high-dose group, compared to controls. Hematology values were not affected.

Necropsy of animals that survived the study demonstrated no consistent pattern of gross pathology effects. Histopathology demonstrated no significant incidence of neoplasms related to treatment. There was a slightly significant increase in the incidence of keratoacanthoma in female rats, but this effect does not appear to be biologically significant.

Determination of plasma drug concentrations demonstrated sytemic exposure that was not consistently dose-proportional. On study day 453, mean plasma concentrations (within sex groups) were not significantly different in the three dose groups. The following average plasma drug concentrations were determined on day 453 in males and females, respectively: 62.05 ± 10.21 µg/mL and 91.76 ± 32.26 µg/mL (20 mg/kg/day), 77.49 ± 20.99 µg/mL and 132.53 ± 21.20 µg/mL (100 mg/kg/day), 92.02 ± 19.46 µg/mL and 132.60 ± 23.72 µg/mL (500 mg/kg/day).

Comment: The average steady-state plasma concentration of atovaquone observed in humans given therapeutic doses (750 mg twice daily) was 22.0 ± 10.1 µg/mL. Systemic exposures achieved in rats were thus ~ 2.8- to 6-fold greater than average clinical exposure. A maximum tolerated dose was clearly not achieved in this study. However, it appears doubtful that significant increases in systemic exposure would have been achieved at a dose higher than 500 mg/kg/day. The study is acceptable (it should be noted that the study design was not reviewed by the Executive CAC).

**EVALUATION AND CONCLUSION**

The statistical analysis of this study was reviewed by Dr. Thomas Hammerstrom. No formal review was provided by Dr. Hammerstrom, but he commented in an electronic mail message that the sponsor's analysis was adequate.

No regulatory action is indicated.

Kenneth L. Hastings, Dr.P.H.

**concurrences:**
HFD-530/ADDir/WDempsey
HFD-530/TL/JFarrelly
KENNETH L. HASTINGS/Pharm/3-26-97

**disk:**
HFD-530/JFarrelly

**cc:**
HFD-530 (N-085)
HFD-530 Division File
HFD-340
HFD-530/CSO/BAtkins
HFD-530/Pharm/KHastings
HFD-530/MO/DBirnkrant
HFD-530/Chem/PLiu
HFD-530/Micro/SBala

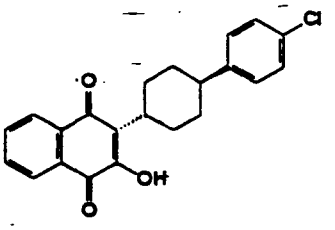
**PHARMACOLOGIST'S REVIEW**

**IND #:** [redacted]

**DATE SUBMITTED:** June 20, 1996
**DATE ASSIGNED:** June 20, 1996
**DATE REVIEW COMPLETED:** March 26, 1997
**HFD-530**

**SPONSOR:** Glaxo Wellcome Inc.
Five Moore Drive
Research Triangle Park, NC 27709

**DRUG:** Atovaquone (Mepron®; 566C80)
**CAS REGISTRY #:** 95233-18-4
**CHEMICAL NAME:** *trans*-2-[4-(4-chlorophenyl)cyclohexyl]-3-hydroxy-1,4-naphthalenedione
**CHEMICAL STRUCTURE:**




**RELATED DOCUMENT:** NDA 20-500

**INDICATION:** Treatment of *Pneumocystis carinii* pneumonia (PCP)

**BACKGROUND**

Atovaquone is a marketed drug product used in treating HIV-infected patients with PCP. As part of Phase IV commitments made by the sponsor, rodent carcinogenicity studies were conducted. This submission contains the report of an oral carcinogenicity study which was conducted in mice.

**NONCLINICAL TOXICOLOGY**

*An oral carcinogenicity study with 566C80 in* [redacted] *CD-1® mice*
*Study # TTEP/95/0083; study dated 5-13-96*

[75/sex/dose (with 2 dietary control groups), 750 total; 36/sex/dose for plasma drug level determinations, 216 total; dietary administration for 2 years (740 - 743 consecutive days for males, 728 - 730 consecutive days for females); doses used: 50, 100, 200 mg/kg/day; performed by the sponsor; drug lot # 566C80WS (reference # 89/0013-003-4), 566C80WAJ (reference # 90/0001-022); dietary controls; GLP study]

The sponsor determined drug concentrations in diet admixtures after preparation of each batch (for the first 13 weeks), every month therafter, and at the end of the study. The mice were

approximately 39 days (males) and 43 days (females) of age at the start of the study.

The following survival rates (expressed as percentages) were observed at the end of the study (with doses given in parentheses): (males), 39 (0), 41 (0), 48 (50), 40 (100), 19 (200); (females), 36 (0), 40 (0), 33 (50), 31 (100), 32 (200). A statistically significant ($p \leq 0.01$) decrease in survival was observed only in high-dose group males. The only treatment-related clinical sign observed on study was yellow and/or pink staining of the pelage due to the color of the drug. There were no effects of treatment on body weight gains or food consumption. Hematology values were not affected.

Necropsy of animals that survived for two years demonstrated enlarged livers in all treatment groups, dose-related increases in discoloration and/or raised areas on livers, increased incidences of lobulated livers (mid- and high-dose group animals), and increased incidences of liver masses or nodules (all treatment groups). Histopathology demonstrated primarily liver effects, including hepatocellular hypertrophy and necrosis (individual cell and diffuse), in all treatment groups. Hepatic regenerative hyperplasia was observed in high-dose group mice.

There was a statistically significant ($p \leq 0.01$) increased incidence of hepatocellular adenoma in males in all treatment groups, compared to either control group or both combined. The effect was not dose-related. The following incidence ratios of hepatocellular adenomas were seen in males at terminal sacrifice (with doses given in parentheses): 5/29 (0), 7/29 (0), 23/34 (50), 16/27 (100), 7/13 (200). In females, there were statistically significant increases ($p \leq 0.01$) in hepatocellular adenoma, carcinoma, and adenoma/carcinoma combined in the mid- and high-dose groups (compared to controls, both individual and combined). In addition, the combined incidence of hepatocellular adenoma and carcinoma was significantly increased in females in the low-dose group when compared to either one control group or both combined. The following incidence ratios of hepatocellular adenomas were seen in females at terminal sacrifice (with doses given in parentheses): 0/27 (0), 2/29 (0), 1/24 (50), 10/20 (100), 6/20 (200). The following incidence ratios of hepatocellular carcinomas were seen in females at terminal sacrifice: 0/27 (0), 0/29 (0), 2/24 (50), 4/20 (100), 4/20 (200).

The following incidences of hepatocellular adenomas were observed in males or females, respectively, found dead on study: 6/42 0/46 (0), 2/38 0/43 (0), 8/40 3/41 (50), 21/41 11/50 (100), 15/58 13/50 (200). The following hepatocellular carcinoma rates were observed: 5/42 0/46 (0), 4/38 0/43 (0), 7/40 0741 (50), 9/41 3/50 (100), 8/58 2/50 (200). Rates of either hepatocellular

adenomas or carcinomas were not increased compared to controls in animals sacrificed moribund on study. No other pathological effects appeared to be significantly associated with treatment.

Determination of plasma drug concentrations demonstrated dose-proportional systemic exposure in the 50 and 100 mg/kg/day groups. Systemic exposure in the 200 mg/kg/day group was generally less than observed in the 100 mg/kg/day group. The following average plasma drug concentrations were determined on day 367 in males and females, respectively: 39.10 ± 16.60 µg/mL and 30.63 ± 7.57 µg/mL (50 mg/kg/day), 78.72 ± 8.12 µg/mL and 62.67 ± 8.55 µg/mL (100 mg/kg/day), 66.31 ± 19.02 µg/mL and 55.99 ± 5.61 µg/mL (200 mg/kg/day). Similar values were obtained at the end of the study.

**Comment:** Although tumor rates did not appear to be dose-related, the incidences may have been exposure related. The average steady-state plasma concentration of atovaquone observed in humans given therapeutic doses (750 mg twice daily) was 22.0 ± 10.1 µg/mL. Thus, increased incidences of hepatocellular adenomas and carcinomas were seen in mice at systemic drug exposures of ~ 1.4- to 3.6-fold greater than average clinical exposure.

**EVALUATION AND CONCLUSION**

The statistical analysis of this study was reviewed by Dr. Thomas Hammerstrom. Dr. Hammerstrom did not write a formal review, but commented in an electronic mail message that "...the report fulfills standards and doesn't require any further statistical review."

The sponsor included several comments concerning the findings in this study. Atovaquone is not genotoxic. However, it is hepatotoxic in mice and is known to produce liver necrosis with compensatory regenerative hyperplasia. In addition, atovaqoune is known to induce cytochrome $P_{450}$ 2B1 activity, which has been associated with tumorigenesis in mice. It is unclear if this effect is predictive of human susceptibility to cancer.

Atovaquone is used for a non-trivial, frequently life-threatening, indication. Based on results of this study, a change in product labelling is required. No other regulatory action is needed.

/S/

/Kenneth L. Hastings, Dr.P.H.

concurrences:
HFD-530/ADDir/WDempsey
HFD-530/TL/JFarrelly
KENNETH L. HASTINGS/Pharm/3-26-97

disk:
HFD-530/JFarrelly

cc:
HFD-530 (N-083)
HFD-530 Division File
HFD-340
HFD-530/CSO/BAtkins
HFD-530/Pharm/KHastings
HFD-530/MO/DBirnkrant
HFD-530/Chem/PLiu
HFD-530/Micro/SBala

JUN 21 1996

**Review and Evaluation of Pharmacology and Toxicology Data**
**Division of Anti-Infective Drug Products, HFD-520**

**IND:** [redacted]

**DRUG:** Malarone Tablets

**CATEGORY:** Anti-malarial

**SPONSOR:** Glaxo Wellcome
5 Moore Drive
Research Triangle Park, N.C. 27709

**CONTACT PERSON:** Thomas Shumaker, Manager, Regulatory Affairs
Phone (919) 483-9324

**NUMBER OF VOLUMES:** 16

**DATE OF SUBMISSION:** March 25, 1996

**DATE CDER RECEIVED:** March 27, 1996

**DATE ASSIGNED:** April 2, 1996

**DATE REVIEW STARTED:** May 7, 1996

**DATE FIRST DRAFT COMPLETED:** June 14, 1996

**DATE REVIEW ACCEPTED BY SUPERVISOR:** June 19, 1996

**INTRODUCTION/OVERVIEW**

Malarone tablets are a fixed dose combination of two marketed products, atovaquone and proguanil, in a ratio of 2.5 to 1. Atovaquone is also known by the code number 566C80 and by the trade name Mepron. Proguanil is also known as chloroguanide, and has the code number 336U50, and the trade name Paludrine.

Atovaquone has been used for more than two years to treat certain infections in AIDS patients. Proguanil has been used since the 1950s for the prophylaxis and treatment of malaria. Previous experience in humans has shown that with each of these agents, the incidence of side effects has been relatively low.

In recent years, resistance has developed to several individual antimalarial agents. Because of the resistance problem, most of the current therapeutic regimens employ a combination of two

antimalarial agents. The two agents in this combination act by different mechanisms. Atovaquone inhibits the electron transport system at the level of the cytochrome bc1 complex. Proguanil inhibits folate metabolism at the level of dihydrofolate reductase.

The Malarone combination has been evaluated in previous clinical trials in Africa and Asia, and has achieved excellent cure rates. The purpose of this IND is to support a Phase II clinical evaluation of this combination in volunteers who are at risk of developing *Plasmodium falciparum* malaria. The trial will be conducted in Kenya, and the principal investigator, Dr. Dennis Shanks, is a United States Army doctor.

This IND contains reports of three toxicology studies on the combination of atovaquone and proguanil, along with a summary of previously submitted data on atovaquone and a review of the published literature on proguanil. The submission also contains pharmacokinetic information on the combination, and on each of the individual agents. Summaries of the pertinent pharmacokinetic data from the one-month rat and dog studies with the combination can be found in Tables 1-4 (attached to this review). The toxicology studies are reviewed in the next section.

**PRECLINICAL SAFETY STUDIES**

The toxicity of atovaquone had previously been evaluated in a comprehensive battery of toxicology studies. The results of these studies are summarized in Table 5 (attached).

The toxicity of proguanil was studied in several animal species in the late 1940s. The results of the acute studies are presented in Table 6. In general, the compound appeared to be more toxic in the smaller species of animals, and less toxic in larger species. The results of the multiple dose studies were not described in extensive detail. The three studies described below deal with the combination. Lot numbers for the test substances were not presented, however "reference numbers" were given.

**1. One Month Oral Toxicity Study in Rats**

This was a GLP study conducted by the sponsor in North Carolina during September-October, 1994 (study 94/0106). Sprague-Dawley rats were maintained under appropriate environmental conditions (food, water, temperature, humidity, lighting, etc.). Atovaquone and proguanil were suspended separately in 0.25% methylcellulose, and administered orally to the rats, according to the following experimental design:

| Group | Number of Animals (M/F) | Dose of Atovaquone (mg/kg/day) | Dose of Proguanil (mg/kg/day) | Volume (ml/kg) |
|---|---|---|---|---|
| 1 (Control) | 15/15 | 0 | 0 | 10 |
| 2 | 10/10 | 20 | 8 | 5+5 |
| 3 | 10/10 | 100 | 40 | 5+5 |
| 4 | 15/15 | 500 | 200 | 5+5 |
| 5 | 15/15 | 500 | 0 | 5 |
| 6 | 15/15 | 0 | 200 | 5 |
| 7 | 12/12 | 20 | 8 | 5+5 |
| 8 | 12/12 | 100 | 40 | 5+5 |
| 9 | 12/12 | 500 | 200 | 5+5 |
| 10 | 12/12 | 500 | 0 | 5 |
| 11 | 12/12 | 0 | 200 | 5 |

The animals were seven weeks old at the start of the study. Five animals of each sex from groups 1, 4, 5, and 6 were placed on a recovery phase (14 days) at the end of dosing. The animals in groups 7, 8, 10, and 11 were used for the determination of plasma drug concentrations. Because of toxicity in group 9, these animals were sacrificed, and replaced with additional animals that were dosed with proguanil alone (40 mg/kg) and used for toxicology measurements. The dose in group 11 was also reduced to 40 mg/kg.

Evaluations for treatment-related effects were based on observations, body weights, food consumption, eye examinations, hematology, serum chemistry, urinalysis, organ weights, gross pathology, and microscopic histopathology.

The purity of the bulk drug, and the potency and stability of the dosing suspensions were established by analysis. Some of the atovaquone suspensions assayed at only ~~—~~ of label claim.

Mortalities occurred in the groups that received proguanil at 200 mg/kg (with or without atovaquone). Deaths also occurred in the group that had received proguanil (40 mg/kg) plus atovaquone (100 mg/kg).

Unkempt appearances and decreased activities were observed in the animals that had received proguanil at 40 mg/kg (with or without atovaquone). Increases in SGOT, SGPT, and BUN occurred in these groups. Increased adrenal and liver weights (absolute and relative) were also seen in these groups. Microscopically, loss of epithelium was seen in cecum and gastric mucosa of these animals. In the kidney, dilated tubules, mineralized casts, and regenerative tubular epithelium were observed microscopically. The microscopic changes were reversible during the recovery period.

No treatment-related toxicity was seen in the groups that had received atovaquone at 20 mg/kg plus proguanil at 8 mg/kg, or in the groups that had received atovaquone alone at 500 mg/kg.

**2. One Month Oral Toxicity Study in Dogs**

This was a GLP study conducted by the sponsor in North Carolina during September-November, 1994 (study 94/0105). Beagle dogs were maintained under appropriate environmental conditions (food, water, temperature, humidity, lighting, etc.). Atovaquone and proguanil were suspended separately in 0.25% methylcellulose, and each individual dose was placed in a gelatin capsule, and administered orally to the dogs, according to the following experimental design:

| Group | Number of Animals (M/F) | Dose of Atovaquone (mg/kg/day) | Dose of Proguanil (mg/kg/day) |
|---|---|---|---|
| 1 | 4/4 | 0 | 0 |
| 2 | 2/2 | 20 | 8 |
| 3 | 2/2 | 50 | 20 |
| 4 | 4/4 | 100 | 40 |
| 5 | 4/4 | 100 | 0 |
| 6 | 4/4 | 0 | 40 |

The animals were 13-18 months of age at the start of the study. Two animals of each sex from groups 1, 4, 5, and 6 were placed on a recovery phase (14 days) at the end of dosing.

Evaluations for treatment-related effects were based on observations, body weights, food consumption, eye examinations, electrocardiograms, hematology, serum chemistry, urinalysis, organ weights, gross pathology, and microscopic histopathology.

The purity of the bulk drug, and the potency and stability of the dosing suspensions were established by analysis.

Three animals in the group that received proguanil alone (40 mg/kg) were sacrificed moribund. Two dogs that received atovaquone (100 mg/kg) plus proguanil (40 mg/kg) were found dead. There were no deaths at lower doses, or in the group that received atovaquone alone. The signs seen prior to death included salivation, emesis, labored breathing, tremors, decreased activity, and prostration. Some of these signs were also seen in the surviving animals from these groups.

The other signs of toxicity that were seen in the groups that received proguanil at 40 mg/kg (groups 4 and 6) were decreases in food consumption and body weight, increases in some hematology and serum chemistry parameters (red blood cells, creatinine, blood urea nitrogen, alkaline phosphatase, glucose, total bilirubin, sodium, potassium), increased relative adrenal weights, gross changes (ulcerations on the lips, stomach, and intestines), and microscopic changes (erosions or ulceration of the tongue, buccal mucosa, esophagus, stomach, small and large intestine, renal tubular necrosis, hypocellular bone marrow, splenic involution, thymic and lymph node atrophy). Most of the changes were reversible during the recovery period.

**3. Oral Teratology Study in Rats**

This was a GLP study conducted by the sponsor in North Carolina during December, 1994 to March 1995 (study 95/0036). Nulliparous female CD rats were mated with males, and were maintained under appropriate environmental conditions (food, water, temperature, humidity, lighting, etc.). Atovaquone and proguanil were suspended separately in 0.25% methylcellulose, and administered orally by gavage once daily during gestation days 6-17, according to the following experimental design:

| Group | Number of Dams | Dose of Atovaquone (mg/kg/day) | Dose of Proguanil (mg/kg/day) |
|---|---|---|---|
| 1 | 30 | 0 | 0 |
| 2 | 30 | 12.5 | 5 |
| 3 | 30 | 25 | 10 |
| 4 | 30 | 50 | 20 |
| 5 | 30 | 50 | 0 |
| 6 | 30 | 0 | 20 |

Each suspension was administered in a volume of 2.5 ml/kg. The rats were 11-12 weeks old at the start of dosing. Observations, body weights, and food consumption measurements were made on the dams. On gestation day 20, all dams were sacrificed, and uterine weights, number of corpora lutea, implantation sites, live and dead fetuses, and early and late resorptions were recorded. Each live fetus was weighed, sexed, examined for external malformations, and the crown-rump distance was measured. About one-half of the fetuses in each litter were dissected and examined for visceral anomalies and for brain or eye abnormalities. All fetuses were cleared, stained with Alizarin red, and examined for skeletal defects.

The purity of the bulk drug, and the stability of the dosing suspensions were established by analysis. Some of the atovaquone dosing suspensions assayed at only — of label claim.

There were four deaths in dams dosed with proguanil at 20 mg/kg (two in group 4, and two in group 6). Salivation was also observed in all proguanil-treated groups.

No effect was observed on pregnancy rate, litter size, dead or resorbed fetuses, post-implantation loss, or fetal body measurements. Some relatively minor anomalies occurred in all groups, including the control group, but the incidence did not appear to be treatment-related. It was concluded that these two compounds were not teratogenic at these doses (alone or in combination).

## CONCLUSIONS/RECOMMENDATIONS TO SPONSOR

In the one-month studies in rats and dogs, mortalities and other signs of toxicity (mainly renal and gastrointestinal effects) were observed in those groups of animals that received proguanil at doses of 40 mg/kg/day or higher (either alone or in combination with atovaquone). Four deaths also occurred in pregnant rats that had received proguanil at 20 mg/kg/day. These doses are higher than the doses that will be used in the clinical trials. Most of the toxicity produced by the combination, appears to be due to proguanil.

Proguanil has been used in humans for many years, without reports of excessive adverse reactions. Atovaquone has been used in immunocompromised patients, also without reports of excessive adverse reactions. Limited clinical experience with the Malarone combination, has suggested that the tablets will be well-tolerated. It is considered to be reasonably safe to initiate this clinical trial.

/S/

Kenneth Seethaler, Ph.D., D.A.B.T.
Pharmacologist, HFD-520

cc: Original IND
HFD-340
HFD-520
HFD-520/Pharm/K.Seethaler
HFD-520/MO/P.Coyne
HFD-520/Micro/J.King
HFD-520/Chem/D.Katague
HFD-520/CSO/P.Fogarty

Concurrence Only:
HFD-520/L.Gavrilovich /S/
HFD-520/R.Osterberg

/S/

Keywords
malarone
atovaquone
proguanil
mepron
paludrine
chloroguanide
deaths
renal toxicity
gastrointestinal tract toxicity

**Table 1. Atovaquone Plasma $C_{max}$ and AUC Values After Repeat Dose Administration of Atovaquone/Proguanil to Rats**

| | atovaquone 20: proguanil 8 (mg/kg/day) | | atovaquone 100:proguanil 40 (mg/kg/day) | | atovaquone 100 (mg/kg/day) | |
|---|---|---|---|---|---|---|
| | Day 1 | Day 29 | Day 1 | Day 29 | Day 1 | Day 29 |
| AUC (µg/mL x h) | 506 | 946 | 920 | 2029 | 1073 | 1358 |
| Cmax (µg/mL) | 27.8 | 47.8 | 55.4 | 97 | 61.6 | 66.1 |

**Table 2. Proguanil Plasma $C_{max}$ and AUC Values After Repeat Dose Administration of Atovaquone/Proguanil to Rats**

| | atovaquone 20: proguanil 8 (mg/kg/day) | | atovaquone 100:proguanil 40 (mg/kg/day) | | proguanil 40 (mg/kg/day) | |
|---|---|---|---|---|---|---|
| | Day 1 | Day 29 | Day 1 | Day 29 | Day 1 | Day 29 |
| AUC (µg/mL x h) | not estimated | not estimated | not estimated | 0.94 | not estimated | 1.12 |
| Cmax (µg/mL) | <0.05 | <0.05 | 0.11 | 0.22 | 0.09 | 0.27 |

**Table 3. Atovaquone Plasma $C_{max}$ and AUC Values After Repeat Dose Administration of Atovaquone/Proguanil to Dogs**

| | atovaquone 20: proguanil 8 (mg/kg/day) | | atovaquone 50: proguanil 20 (mg/kg/day) | | atovaquone 100: proguanil 40 (mg/kg/day) | | atovaquone 100 (mg/kg/day) | |
|---|---|---|---|---|---|---|---|---|
| | Day 1 | Day 29 | Day 1 | Day 29 | Day 1 | Day 29 | Day 1 | Day 29 |
| AUC (µg/mL x h) | 30.9 | 130.7 | 36.0 | 171.5 | 28.7 | 158.1 | 81.4 | 270.8 |
| Cmax (µg/mL) | 3.3 | 8.9 | 3.6 | 10.0 | 2.7 | 9.2 | 7.6 | 23.1 |

**Table 4. Proguanil Plasma $C_{max}$ and AUC Values After Repeat Dose Administration of Atovaquone/Proguanil to Dogs**

| | atovaquone 20: proguanil 8 (mg/kg/day) | | atovaquone 50: proguanil 20 (mg/kg/day) | | atovaquone 100: proguanil 40 (mg/kg/day) | | proguanil 40 (mg/kg/day) | |
|---|---|---|---|---|---|---|---|---|
| | Day 1 | Day 29 | Day 1 | Day 29 | Day 1 | Day 29 | Day 1 | Day 29 |
| AUC (µg/mL x h) | 7.5 | 7.9 | 12.1 | 12.1 | 11.3 | 24.7 | 11.4 | 15.9 |
| Cmax (µg/mL) | 0.9 | 0.8 | 1.7 | 1.1 | 1.3 | 2.1 | 1.3 | 1.6 |

## Table 5. Oral Toxicology Studies Conducted with Atovaquone

| Type | Study | Species | No./Group (M/F) | Dose (mg/kg/day) | Duration | Findings |
|---|---|---|---|---|---|---|
| ACUTE TOXICITY STUDIES | Median Lethal Dose | CD-1 Mouse | 5/5 | 1, 1825 | Single dose | MLD >1825 mg/kg |
| | Median Lethal Dose | Wistar Rat | 5/5 | 1, 1825 | Single dose | MLD >1825 mg/kg |
| SUBCHRONIC TOXICITY STUDIES | Oral Toxicity (90 Days) | CD-1 Mouse | 16/16 | 0, 50, 200, 800 | 90 Days (diet) | Hepatocellular hypertrophy and individual cell necrosis<br>Increased hepatocellular SER |
| | Oral Toxicity (28 Days) | Wistar Rat | 15/15 | 0, 20, 100, 500 | 28 Days (gavage)<br>(14 days postdose follow-up) | No effect on any parameter |
| | Oral Toxicity (90 Days) | CD Rat | 13/13 | 0, 50, 200, 800 | 90 Days (diet) | Equivocal reduction in erythrocytic parameters |
| | Oral Toxicity (6 Months) | Wistar Rat | 30/30 | 0, 20, 100, 500 | 6 Months (gavage)<br>(28 days postdose follow-up) | Equivocal reduction in erythrocytic parameters |
| | Oral Toxicity (12 months) | CD Rat | 25/25 | 0, 20, 100, 500 | 12-month (diet);<br>35 days post-dose follow-up | Minimal, equivocal reduction in erythrocytic parameters |
| | Oral Toxicity (28 Days) | Beagle Dog | 3 or 5/3 or 5 | 0, 20, 100, 500 | 28 Days<br>(14 days postdose follow-up) | No effect on any parameters |
| | Oral Toxicity (6 Months) | Beagle Dog | 6/6 | 0, 20, 100, 500 | 6 Months<br>(28 days postdose follow-up) | No effect on any parameters |
| | Oral Toxicity (12 months) | Beagle Dog | 6/6 | 0, 20, 100, 500 | 12-month (diet);<br>34 days post-dose follow-up | No effect on any parameters. |

## Table 5 Oral Toxicology Studies Conducted with Atovaquone (continued)

| Type | Study | Species | No./Group (M/F) | Dose (mg/kg/day) | Duration | Findings |
|---|---|---|---|---|---|---|
| TERATOLOGY STUDIES | Oral Fetal Toxicity and Teratogenicity | CD Rat | 30 F | 0, 250, 500, 1000 | Gestation days 6 through 15 | Not teratogenic under conditions tested |
| | Oral Fetal Toxicity and Teratogenicity | NZW Rabbit | 25 F | 0, 300, 600, 1200 | Gestation days 6 through 18 | Not teratogenic under conditions tested |
| | Reproduction Infertility Evaluation | CD Rat | 35/35 | 0, 100, 300, 1000 | Male – 73 days prior to mating and up to gestation day 20 Female – 14 days prior to mating and gestation and up to day 20 of lactation. | No effect on reproductive, fertility, or behavioral parameters. |
| | Peri and Post Natal Evaluation | CD Rat | 0/30 | 0, 250, 500, 1000 | Gestation day 15 through postpartum day 20. | No effect on gestation, parturition, or survival of F1 generation. |
| MUTAGENICITY STUDIES | Ames Assay | *Salmonella typhimurium* | NA | Up to 79 µg/plate | NA | Nonmutagenic |
| | Mouse Lymphoma Assay | Mouse | NA | Up to 50 µg/mL | NA | Nonmutagenic |
| | *In Vitro* Cytogenetic Assay | Human Lymphocyte | NA | 15, 30, 50 µg/mL (with S9) 2.5, 5, 10 µg/mL (without S9) | NA | No chromosomal abberations |
| | Micronucleus Assay | Mouse | 5/5 | 1000, 3000, 5000 | Single dose | No chromosomal abberations |
| SUBCHRONIC TOXICITY STUDIES (SUSPENSION) | Oral Toxicity (90 days) | CD Rat | 14/4 | 0, 20, 100, 200, 500 | 98 Days (Gavage) (13 days post-dose follow-up) | Equivocal increase in reticulocytes |
| | Oral Toxicity (30 days) | Beagle Dog | 5/5 | 0, 20, 100, 200, 500 | 33 Days (23 days post-dose follow-up) | Slight decrease in erythrocytic parameters |
| | Oral Toxicity (90 days) | Beagle Dog | 5/5 | 0, 20, 100, 200, 500 | 92 Days (14 days post-dose follow-up) | Marginal decrease in erythrocytic parameters and slight increase in reticulocytes |

Table 6. Acute Toxicity of Proguanil

| Species | Route of Administration | Acute $LD_{50}$[a] (mg/kg) |
|---|---|---|
| Mouse | Oral | 23 |
| | Intramuscular | 20 |
| | Intraperitoneal | 20 |
| | Intravenous | 20 |
| Rat | Oral | 200 |
| | Intraperitoneal | 40 |
| | Intravenous | 40 |
| Rabbit | Oral | 150 |
| | Intraperitoneal | 50 |
| Dog | Oral | >400 (no deaths) |
| | Intramuscular | *ca.* 120 |
| Rhesus monkey | Oral | >400 (no deaths) |
| | Intramuscular | *ca.* 120 |

[a] Lowest value reported