National Institute of
Environmental Health Sciences

NIEHS Technical Report on the Reproductive, Developmental, and General Toxicity Studies of 3′-Azido-3′-deoxythymidine (AZT) and Clarithromycin Combinations (CAS Nos. 30516-87-1 and 81103-11-9) Administered by Gavage to Swiss CD-1® Mice

AIDS 09

October 2003

NIEHS AIDS Therapeutics
Toxicity Report
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NIH Publication No. 04-4418
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U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health

FOREWORD

Infection with human immunodeficiency virus (HIV) causes immunosuppression and leads to acquired immunodeficiency syndrome (AIDS) with a broad spectrum of opportunistic infections. Prophylaxis and treatment of AIDS are generally combination therapies of antiretroviral agents with antimicrobial drugs specific for the opportunistic infections. The National Institute of Environmental Health Sciences (NIEHS), under the AIDS research program, is evaluating AIDS therapeutics for reproductive, developmental, and general toxicity in rodents. These evaluations may include single therapeutic agents or combination therapies when the toxic potential of these agents in animal models is not available or is incomplete.

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CONTRIBUTORS

This report on the reproductive, developmental, and general toxicity studies of 3'-Azido-3'-deoxythymidine (AZT) and clarithromycin combinations in Swiss (CD-1®) mice is based primarily on studies that began in November 1996 and ended in July 1997 at Southern Research Institute, Birmingham, AL.

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PEER REVIEW

The draft report on the reproductive, developmental, and general toxicity studies of 3'-azido-3 '-deoxythymidine (AZT) and clarithromycin combinations will be evaluated by reviewers. These reviewers serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determine if the design and conditions of the study are appropriate and ensure that this reproductive, developmental, and general toxicity studies report presents the experimental results and conclusions fully and clearly. The comments of the reviewers will be reviewed prior to finalization of this document. Changes will be made such that the concerns of the reviewers will be addressed to the extent possible.

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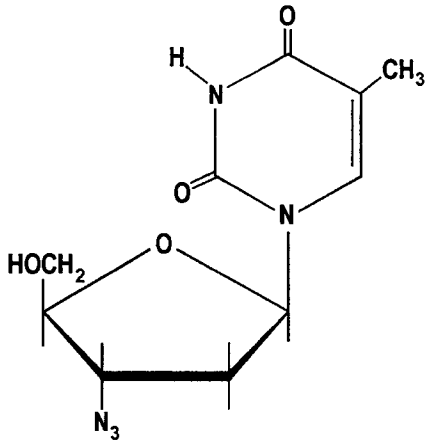
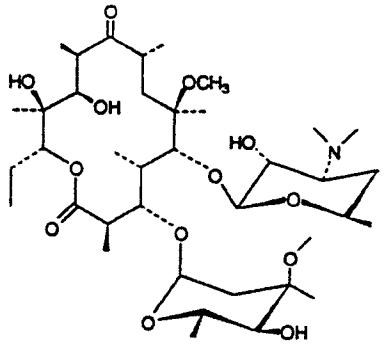
CONTENTS

ABSTRACT

3′-Azido-3′-deoxythymidine (AZT) and Clarithromycin Combinations



AZT
Molecular Formula: $C_{10}H_{13}N_5O_4$
Molecular Weight: 267.24
CAS Number: 30516-87-1



Clarithromycin
Molecular Formula: $C_{38}H_{69}NO_{13}$
Molecular Weight: 747.97
CAS Number: 81103-11-9

Two study protocols were used to evaluate the reproductive, developmental, and general toxicity of 3′-azido-3′-deoxythymidine (AZT) and clarithromycin in Swiss (CD-1®) mice treated by oral gavage. For both studies, male mice (10 to 18/group) were dosed from study day 5 until the day prior to sacrifice on study day 25 or 26. Females were divided into two groups designated female-A and female-B mice. The female-A mice (20 to 28/group) were dosed from day 0 to sacrifice. They were cohabited with treated males on days 9 to 13 to test for effects on mating behavior, fertilization, and implantation. Caesarean sections were performed on presumed day 18 of gestation (days 28-32). The female-B mice (approximately 20/group) were cohabited with untreated males on days 0 to 4. Sperm-positive female-B mice were dosed during organogenesis on days 6 to 15 of presumed gestation and sacrificed on day 4 of lactation.

In the initial study, doses of clarithromycin (500, 1,250, or 2,500 mg/kg) were approximately 2, 5, or 10 times the human therapeutic dose. The formulations contained numerous excipients included by the manufacturer in the tablet preparation of this antibiotic for human therapeutic use. Excessive mortality occurred in male and female groups treated with 1,250 or 2,500 mg/kg of clarithromycin alone and in combination with AZT; mortality was

100% in male and female-A groups receiving 2,500 mg/kg. The mortality was attributed to a combination of toxicity in multiple vital organs and chronic dilatation of the stomach from the large volume of thick gavaged material resulting in localized complications (fungal infection and inflammation). Because of excessive mortality, a second study was conducted using 250, 500, and 1,000 mg/kg purified clarithromycin, equivalent to approximately 1, 2, and 4 times the human therapeutic dose, and 200 and 400 mg/kg AZT, which are approximately 2 and 4 times the human therapeutic dose.

The most significant effects of treatment with 200 or 400 mg/kg AZT and 250, 500, or 1,000 mg/kg purified clarithromycin are summarized here. Administration of AZT alone resulted in slight hematopoietic toxicity manifested by mild declines in red blood cell, hemoglobin, and hematocrit values. Hematopoietic cell proliferation and increased hemosiderin deposition in the spleen accompanied the mild alterations in erythrocyte parameters. Administration of 250 or 500 mg/kg clarithromycin alone did not result in significant toxicity. Administration of 1,000 mg/kg clarithromycin alone resulted in multiple organ toxicity (liver, spleen, bone marrow, kidney, brain, heart, lymph nodes, and thymus) in a few male and female-A mice. Cytoplasmic vacuolization of phagocytic cells occurred in the majority of tissues with lesions. A slight decline in body weight, slight leukocytosis, neutrophilia, and lymphocytosis occurred in the high dose female-A group.

Administration of AZT in combination with 250, 500, or 1,000 mg/kg clarithromycin resulted in hematopoietic toxicity in male and female-A mice, and the severity of the anemia was far greater than that induced by AZT alone. The anemia was accompanied by bone marrow depletion and by hematopoietic cell proliferation and hemosiderin deposition in the spleen. The anemia was macrocytic in the majority of the treatment groups but progressed to a microcytic anemia in the highest dose female-A group. Reticulocytopenia and sporadic thrombocytosis were also considered to be treatment-related manifestations of hematopoietic toxicity. In general, alterations in other tissues were similar in morphology, incidence, and severity to those induced by clarithromycin alone.

Reproductive and developmental effects occurring after treatment with AZT alone included a slight decline in pregnancy rate, reduced live litter size, increased numbers of resorptions, and a slight decline in total weight per litter. Treatment with clarithromycin alone resulted in lower fertility with reductions in pregnancy rate, the number of litters delivered, and litter size. Fertility was further reduced in groups treated with combinations of AZT and clarithromycin. Combination therapy resulted in reduced pregnancy rates, reduced live litter size, increased numbers of resorptions, and declines in fetal and pup weights per litter. Fewer pups survived to postnatal day 4.

Summary of Significant Treatment-Related Toxicological Parameters in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations in Swiss (CD-1®) Mice

Treatment Regimen	Male Mice	Female-A Mice	Female-B Mice
Body Weight			
AZT Alone *200 or 400 mg/kg*	no body weight change	decreased body weight	no body weight change
Clarithromycin Alone *250, 500, or 1 000 mg/kg*	no body weight change	decreased body weight	no body weight change
AZT + Clarithromycin *200 or 400 mg/kg +250, 500, or 1,000 mg/kg*	no body weight change	decreased body weight	decreased gestational and lactational body weights
Clinical Pathology			
AZT Alone *200 or 400 mg/kg*	mild decreases in RBC, HGB, and HCT mild increases in MCV and RDW	mild decrease in RBC mild increases in MCV and RDW	mild increases in MCV and RDW
Clarithromycin Alone *250, 500, or 1,000 mg/kg*	no significant change	leukocytosis neutrophilia lymphocytosis	no significant change
AZT + Clarithromycin *200 or 400 mg/kg +250, 500, or 1,000 mg/kg*	anemia increased MCV increased RDW thrombocytosis	anemia increased MCV in lower dose groups decreased MCV in higher dose groups increased RDW reticulocytopenia thrombocytosis mild increase in BUN	increased MCV increased RDW
Histopathology			
AZT Alone *200 or 400 mg/kg*	Liver cytoplasmic alteration (slight, low incidence) Spleen hematopoietic cell proliferation (slight) hemosiderosis (slight)	Liver cytoplasmic alteration (slight, low incidence) Spleen hematopoietic cell proliferation (slight) hemosiderosis (slight)	Histopathological evaluations were performed only on gross lesions
Clarithromycin Alone *250, 500 or 1,000 mg/kg*	Liver cytoplasmic alteration (slight, low incidence) cytoplasmic vacuolization (low incidence) Spleen hematopoietic cell proliferation (slight, low incidence) Kidney nephropathy (low incidence)	Liver cytoplasmic alteration (slight) cytoplasmic vacuolization (low incidence) hepatocyte necrosis (low incidence) Spleen hematopoietic cell proliferation (slight) red pulp atrophy (low incidence) lymphoid follicle depletion (low incidence) Bone marrow depletion (low incidence) Kidney nephropathy (low incidence)	Histopathological evaluations were performed only on gross lesions

(continued)

Summary of Significant Treatment-Related Toxicological Parameters in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations in Swiss (CD-1®) Mice

Treatment Regimen	Male Mice	Female-A Mice	Female-B Mice
Histopathology (continued)			
AZT + Clarithromycin *200 or 400 mg/kg* *+250 500, or* *1 000 mg/kg*	Liver cytoplasmic alteration (slight, low incidence) cytoplasmic vacuolization (low incidence) Spleen hematopoietic cell proliferation hemosiderosis red pulp atrophy (low incidence) lymphoid follicle depletion (low incidence) Bone marrow depletion (low incidence) Kidney nephropathy (low incidence)	Liver cytoplasmic alteration (slight) hepatocyte necrosis (low incidence) cytoplasmic vacuolization (low incidence) Spleen hematopoietic cell proliferation hemosiderosis red pulp atrophy lymphoid follicle depletion (low incidence) Bone marrow depletion Kidney nephropathy	Histopathological evaluations were performed only on gross lesions
Reproductive/Developmental			
AZT Alone *200 or 400 mg/kg*	no adverse effects	reduced live litter size increased number of resorptions slight decline in fetal weight per litter reduced pregnancy rate (400 mg/kg group)	slight decline in number of pups surviving to postnatal day 4 slight increase in number of dams with stillborn pups (400 mg/kg group)
Clarithromycin Alone *250 500 or* *1 000 mg/kg*	no adverse effects	slight reduction in live litter size (1,000 mg/kg group) reduced pregnancy rate (1,000 mg/kg group)	slight reduction in live litter size (1,000 mg/kg group) slight increase in number of dams with stillborn pups (1,000 mg/kg group)
AZT + Clarithromycin *200 or 400 mg/kg* *+250, 500, or* *1,000 mg/kg*	no adverse effects	reduced pregnancy rate reduced live litter size increased number of resorptions prominent decline in fetal weight per litter	decreased number of litters delivered in higher dose groups decreased live litter size decreased number of pups surviving to postnatal day 4 reduced pup weight/litter increased number of dams with stillborn pups

INTRODUCTION

Acquired immunodeficiency syndrome (AIDS) is a lethal multisystem disease that has become a major public health problem since its recognition in 1981 (Gottlieb *et al.*, 1981; Masur *et al.*, 1981; Siegle *et al.*, 1981). The etiological agent of AIDS is a retrovirus that is now referred to as the human immunodeficiency virus (HIV) (Coffin, 1986). To date, the most effective single agent in the treatment of HIV has been the first dideoxynucleoside analogue used in clinical trials, zidovudine (3′-azido-3′-deoxythymidine, AZT, Retrovir, azidothymidine, compound S, BW A509U, CAS No. 30516-87-1), commonly referred to as AZT (Vince *et al.*, 1988; Amin, 1989).

AZT therapy produces numerous beneficial effects in AIDS patients, including decreases in morbidity and increases in lifespan (Amin, 1989; Jeffries, 1989). The most important adverse effects of AZT are anemia and granulocytopenia, which are believed to reflect bone marrow toxicity (Richman, 1988; Amin, 1989). Two types of anemia may occur with AZT therapy: macrocytic megaloblastic anemia and normocytic normochromic anemia.

Several subacute and subchronic rodent toxicity studies have demonstrated that the primary toxicity of AZT is myelosuppression. Male Swiss CD-1® mice were administered 100, 250, 500, or 1,000 mg AZT/kg body weight by gavage for 30 days (Mansuri *et al.*, 1990). No mortality or body weight effects were evident from AZT treatment. Erythropenia and increased mean cell volume were observed at all doses, and anemia was observed at the 1,000 mg/kg dose. Pathologic findings in the AZT-treated mice were consistent with the hematological results and included lymphoid depletion, reticuloendothelial hyperplasia in the spleen and thymus, and bone marrow hypocellularity.

In a 14-week study (NTP, 1999), B6C3F$_1$ mice were treated with 0, 25, 50, 100, 400, or 1,000 mg of AZT/kg body weight administered by gavage twice daily with one half the total dose in 0.5% methylcellulose. On day 5, statistically significant dose-related decreases were observed in reticulocyte counts in males and females. Dose-related anemia was evident on days 23 and 93. To evaluate the ability of treated animals to reverse any compound-related effects when treatment is stopped, additional groups were administered 0, 50, 400, or 1,000 mg/kg AZT twice daily for 14 weeks and then held without additional treatment for 29 days. Improvement of hematology parameters indicated recovery of the bone marrow after treatment stopped. An apparently nontoxic,

treatment-related clinical finding in AZT-treated $B6C3F_1$ mice was a darkening of the skin on the tail, feet, and/or muzzle (Rao *et al.*, 1998).

Oral bioavailability of AZT was determined in female $B6C3F_1$ mice by comparison of the area under the curve obtained from an oral dose to that of an intravenous dose at the same concentration (Trang *et al.*, 1993). Bioavailability was found to be 0.86, 0.78, and 0.97 for the 15, 30, and 60 mg/kg oral doses. The mean elimination half-life values ranged from 17.3 to 19.9 minutes for the three intravenous doses and from 16.5 to 21.9 minutes for the three oral doses. Based on these results, the internal dose of AZT was linear and dose-proportional over the oral dose concentration range administered.

Standard teratology studies of AZT have been performed in rats and rabbits (Ayers, 1988). Rats were dosed orally with 125 to 500 mg/kg on gestation days 6 to 15. No fetal toxicity or teratogenicity was found. Fetal AZT concentrations 30 minutes postdosing were 61 µg/g or 76 times the antiviral ID_{50}. Rabbits were dosed orally at 125 to 500 mg/kg on gestation days 6 to 18, and no fetal toxicity or teratogenicity was found. Fetal AZT concentrations 30 minutes postdosing were 40.2 µg/g, or 50 times the antiviral ID_{50}.

Female Wistar rats were dosed three times orally with 100 mg/kg AZT at 5-hour intervals on gestation day 10 for a total dose of 300 mg/kg (Greene *et al.*, 1990). No adverse effects on maternal body weight gain, feed consumption, hematological parameters, or growth and survival of offspring were observed. AZT concentration measurements 30 minutes after the last dose were 62.6 µg/mL in maternal plasma and 21.1 µg/g in fetal tissue.

Studies in C3H/He mice concluded that AZT has a direct toxic effect on the developing mouse embryo (Toltzis *et al.*, 1991). Female mice were exposed to 0, 0.25, 0.5, or 2.5 mg AZT/mL drinking water for 8 weeks during mating and throughout gestation. All AZT groups had fewer pregnant mice per group, fewer pups per litter, and increased resorptions per mouse. Dose-related embryolethality was observed.

Because AIDS is a disease of immune suppression, the majority of AIDS patients actually die from characteristic opportunistic infections (Hardy, 1991; Harkins and Herriot, 1992). As a result, the treatment of AIDS is increasingly one of combination therapy of anti-HIV drugs and anti-infective drugs (Goldschmidt and Dong, 1992). Disseminated infection with the *Mycobacterium avium* complex (MAC) is the most common cause of bacteremia in AIDS patients, occurring in as many as 50% of these patients (Masur, 1993; Ong, 1999). MAC disease encompasses infections due to the bacterial organisms *M. avium* and *M. intracellulare* and to some strains

of mycobacteria not yet identified (Faris *et al.*, 1998). These organisms are atypical acid-fast bacilli and are usually not pathogenic to the healthy host.

MAC mycobacteria are ubiquitous organisms that can be isolated from water, soil, and a variety of animals, including dogs, cats, chickens, pigs, and insects (Masur, 1993). MAC infection was historically associated with chronic lung disease and received little attention. Infection occurs via respiratory or gastrointestinal routes, and the gastrointestinal tract is thought to be the most common site of colonization and dissemination. Survival of AIDS patients with MAC infection is half that of non-MAC-infected patients.

Disseminated MAC is rarely the initial AIDS-defining illness, because it occurs later in the course of the disease (Faris *et al.*, 1998), and the incidence of MAC infection increases as average survival time increases due to better antiretroviral therapy. The signs and symptoms of MAC infection are nonspecific and include fever, night sweats, diarrhea, abdominal pain, weight loss, anorexia, nausea, vomiting, anemia, and elevated serum alkaline phosphatase. The effect of untreated disseminated disease in AIDS patients is unknown, although several studies have suggested that it is associated with decreased survival (Chaisson *et al.*, 1992).

Current therapeutic recommendations for MAC are that all HIV-infected adults and adolescents with a CD4 lymphocyte count below 50 cells/mm^3 receive clarithromycin at 500 mg twice a day or azithromycin at 1,200 mg once a week (CDC, 1997; Abbott Laboratories, 1998; Wright *et al.*, 1998). Although azithromycin and clarithromycin appear to be similar in efficacy with regard to preventing disseminated MAC, clarithromycin is the only agent currently associated with a survival benefit in a well-controlled trial (Wright *et al.*, 1998). The efficacy of clarithromycin in treatment of disseminated MAC in AIDS patients has been assessed in several clinical trials. Clarithromycin showed significant activity against MAC, and was well tolerated (Dautzenberg *et al.*, 1991a,b, 1992, 1993; Saint-Marc and Touraine, 1991; Gupta *et al.*, 1992). In one early clinical trial, AIDS patients with disseminated MAC receiving twice daily doses of 1,000 mg clarithromycin showed significant decreases in colony-forming units of *M. avium* in blood (Dautzenberg *et al.*, 1991a), and in a second, multicenter trial with 77 AIDS patients, clarithromycin was effective in eradicating the organism from the blood in 63% of patients receiving doses of 500 to 1,000 mg/day and in 98% of patients receiving doses of 1,500 to 2,000 mg/day (Dautzenberg *et al.*, 1993). Patients reported a significant improvement in the quality of life. However, there was a tendency toward relapse after discontinuation of the drug and toward development of resistance by the bacteria after prolonged monotherapy. In a recent risk-benefit assessment of therapies for MAC, Griffith (1999) concluded that monotherapy with clarithromycin at any of the dosages tested was frequently followed by a clarithromycin-resistant isolate. Therefore, as in the standard treatment of infections with *Mycobacterium tuberculosis*, it appears

that monotherapy with clarithromycin for *M. avium* is not adequate due to the development of resistance and that cotreatment with other antibacterial drugs is necessary. One clinical trial of clarithromycin, ciprofloxacin, and amikacin in AIDS patients showed this combination to be effective against MAC infections in patients with bacteremia (Goldman and Longworth, 1993).

The *in vitro* activity of clarithromycin against various strains of mycobacteria has been assessed by numerous investigators (Brown *et al.*, 1992; Heifets *et al.*, 1992; Barradell *et al.*, 1993; Alvarez-Elcoro and Enzler, 1999). Data have consistently shown that clarithromycin is effective at inhibiting the growth of these organisms and that the drug has greater *in vitro* activity against mycobacteria than other similar macrolide antibiotics. Evidence from *in vitro* studies and studies in the beige mouse suggest that clarithromycin may be synergistic with ethambutol and rifampin and additive with clofazimine (Sturgill and Rapp, 1992).

Clarithromycin may also be beneficial in the treatment of encephalitis caused by *Toxoplasma gondii* in AIDS patients (Fernandez-Martin *et al.*, 1991). Infection with the intracellular coccidian protozoan *T. gondii* is a fairly common occurrence in birds and mammals, including humans. Such infections are typically benign and self-limiting in patients with healthy immune systems. However, toxoplasmosis can present a significant hazard to immunocompromised hosts (such as AIDS patients) or when the infection is contracted *in utero* (Saint Georgiev, 1994). More than 3,000 congenital pediatric cases of toxoplasmosis are reported annually. In its most common form in immunodeficient hosts, the disease manifests as toxoplasmic encephalitis, which is fatal if not treated. Toxoplasmic encephalitis is the major cause of focal intracerebral lesions in AIDS patients and results in significant morbidity and mortality in this population. Less commonly, toxoplasmosis can manifest as either a severe interstitial pneumonitis or myocarditis.

Clarithromycin and other macrolides (e.g., spiramycin, roxithromycin) have been shown to be effective against *T. gondii* both *in vitro* and in murine models. In an *in vitro* study using murine macrophages, clarithromycin effectively inhibited the growth of extracellular parasites; however, it was effective against intracellular parasites only at concentrations that were toxic to the host macrophages (Chang and Pechère, 1988). *In vivo*, various investigators have tested the efficacy of clarithromycin alone and in combination with pyrimethamine, minocycline, or sulfadiazine in mice experimentally infected with *T. gondii* (Araujo *et al.*, 1992; Alder *et al.*, 1994). Araujo *et al.* (1992) found that clarithromycin was protective against acute infections with several strains of *T. gondii*, including some isolated from AIDS patients, although the degree of protection varied with different strains of the parasite. Survival was enhanced by cotreatment with clarithromycin and either pyrimethamine or sulfadiazine. In chronically infected mice, cotreatment with clarithromycin and minocycline resulted in greater

activity than either drug alone. In a similar series of experiments, Alder *et al.* (1994) tested the activity of clarithromycin, both alone and in combination with pyrimethamine or minocycline, against *T. gondii* in two strains of mice. Clarithromycin in combination with either pyrimethamine or minocycline was significantly more effective at increasing survival of experimentally infected mice than was any of the three drugs alone. The efficacy of the drug combinations was comparable to the standard pyrimethamine-sulfonamide or pyrimethamine-nonsulfonamide therapy. These authors concluded that measures of the *in vitro* efficacy of clarithromycin were not predictive of *in vivo* efficacy.

Fernandez-Martin *et al.* (1991) tested the efficacy of a clarithromycin-pyrimethamine combination against *T. gondii* in AIDS patients with primary toxoplasmic encephalitis. Patients received 2 g clarithromycin/day and 75 mg pyrimethamine/day for 6 weeks. Complete clinical remission was seen in six of the eight patients that completed the trial. The most common adverse effects were nausea (38%), skin rash (38%), elevated liver enzymes (24%), hearing loss (15%), and hematologic abnormalities, including anemia and thrombocytopenia (31%). In terms of both efficacy and the incidence of adverse effects, the authors considered the clarithromycin-pyrimethamine combination to be comparable to standard therapies. Prophylactic monotherapy with clarithromycin is not currently recommended for toxoplasmic encephalitis (CDC, 1997).

Clarithromycin is a semisynthetic compound that belongs to the class of antibiotics known as macrolides, which are so called because they contain a macrocyclic lactone ring (Barradell *et al.*, 1993). It occurs as a white to off-white crystalline powder and is soluble in acetone, slightly soluble in alcohol, and virtually insoluble in water. Clarithromycin is also known by a variety of other names, including TE-031 and A-56268 (Bahal and Nahata, 1992).

Clarithromycin, like erythromycin and many other macrolides, contains a 14-membered lactone ring. Structurally, it differs from erythromycin in that it contains a methoxy group, instead of a hydroxyl group, at position 6 of the lactone ring. Because of this modification, clarithromycin exhibits significantly improved pharmacokinetic properties and generally lower toxicity than erythromycin and some of the other macrolides. One persistent problem with erythromycin has been its instability under the acidic conditions of the stomach. At acid pH, erythromycin degrades to form first an 8,9-anhydro-6,9-hemiketal, which is then further degraded to erythromycin-6,9; 9,12-spiroketal. Although neither of these degradation products exhibits significant antibacterial activity, the hemiketal is thought to be at least partially responsible for the gastrointestinal irritation that is common in patients taking erythromycin (Kirst and Sides, 1989; Bahal and Nahata, 1992). However, by blocking both hemiketal and spiroketal formation, the 6-methoxy group of clarithromycin confers on this drug a much improved

acid stability. The 6-methoxy substitution has at least two other positive effects in that, besides providing for increased intragastric and intracellular stability of the drug, it also results in a lower incidence of adverse gastrointestinal effects than erythromycin by decreasing the ability of the drug and its metabolites to stimulate gastric motility, and it allows increased oral bioavailability of the drug (Sturgill and Rapp, 1992; Pallasch, 1993).

Clarithromycin is rapidly absorbed following oral administration, with a time-to-maximum-concentration of 1 to 2 hours after a 250 mg dose. Total bioavailability of the drug at this dose is approximately 55% (Goldman and Longworth, 1993). The presence of food in the stomach has been reported to slightly delay absorption (Sturgill and Rapp, 1992), to have no effect on absorption (Pallasch, 1993), or to slightly increase the rate of absorption of the drug (Bahal and Nahata, 1992). In any case, food does not affect total bioavailability of the drug or its metabolites (Abbott Laboratories, 1998).

After absorption, clarithromycin is well distributed in tissues, especially the lung. In mice, studies with radiolabeled clarithromycin showed that it is concentrated in almost all tissues and organs at higher concentrations than in blood and that it is especially prone to accumulate in the lungs, gastrointestinal tract, liver, kidney, and spleen (Kohno *et al.*, 1990a). Uptake into lung cells is believed to be an energy-dependent active transport process (Kohno *et al.*, 1990b). Clarithromycin is primarily metabolized in the liver by the cytochrome P-450 system. The major metabolite is 14-OH-clarithromycin, which has antibacterial activity comparable to the parent compound (Stein and Havlichek, 1992). Minor metabolic pathways, which produce metabolites with little or no biological activity, include N-demethylation and cleavage of the cladinose sugar.

After oral dosing in healthy humans (250 mg twice daily), clarithromycin and its 14-OH metabolite reach peak serum concentrations of 0.8 μg/mL and 0.6 μg/mL, respectively. These serum levels are attained in approximately 1 to 2 hours, and steady state concentrations of both the parent drug and the 14-OH metabolite are reached in 2 to 3 days (Goldman and Longworth, 1993). After a dose of 250 mg every 12 hours, the half-life of elimination is 3 to 4 hours for clarithromycin and 5 to 6 hours for the 14-OH metabolite. This, along with the low gastrointestinal toxicity of the drug, allows for twice daily dosing with clarithromycin, compared to four daily doses that are often required for erythromycin. However, formation of 14-OH-clarithromycin and elimination of clarithromycin and its metabolite exhibit a significant dose-related nonlinearity. Following a 1,200 mg dose of ^{14}C-clarithromycin, total recovery of the 14-OH metabolite was reduced by 40%, and recovery of the parent drug was increased by 75%, compared to a single 250 mg dose (Ferrero *et al.*, 1990). After twice daily dosing at 500 mg/dose, the elimination half-life for the parent drug increased to 5 to 7 hours. This nonlinearity in

metabolism and elimination is thought to be due to saturation of the cytochrome P-450 and secondary metabolic pathways (Sturgill and Rapp, 1992).

Unlike some other macrolides (e.g., azithromycin) that undergo significant concentration in the tissues at the expense of serum levels, clarithromycin maintains relatively high serum concentrations (Stein and Havlichek, 1992). However, although the drug concentrates in tissues, significant accumulation does not occur. In one study, 4 hours after an oral dose of 500 mg in humans, clarithromycin concentrations in the lung were 17.5 mg/kg, compared to 2.5 mg/L in serum. After an additional 8 hours, concentrations had declined to 3.8 mg/kg and 0.4 mg/L for lung tissue and serum, respectively (Franschini *et al.*, 1991a). Similar results have been described for both the parent drug and the 14-OH metabolite using different doses and dosing regimens (Franschini *et al.*, 1991b).

Although both clarithromycin and its 14-OH metabolite are excreted primarily by the kidney, some biliary excretion does occur. After a 250 mg oral dose in adults humans, approximately 20% to 30% is excreted unchanged in the urine. Renal clearance is relatively unaffected by dose and approximates the glomerular filtration rate. However, damage to the kidney can have a profound effect on urinary excretion. Although the half-lives for urinary clearance for the parent and the 14-OH metabolite are 5.8 and 7.2 hours, respectively, when kidneys are functioning normally (creatinine clearance [CL_{cr}] greater than 80 mL/min), these half-lives increase dramatically (9.6 and 16.9 hours at CL_{cr} = 30 to 80 mL/min; and 19.8 and 28.9 hours at CL_{cr} less than 30 mL/min) when kidney function is compromised (Chu *et al.*, 1991).

Several authors have reported on the interactions of clarithromycin with other drugs used in the treatment of AIDS patients (Richens *et al.*, 1990; Albani *et al.*, 1993; Gustavson *et al.*, 1993; Honig *et al.*, 1993; Piscitelli *et al.*, 1996; McConnell and Amsden, 1999; Malaty and Kuper, 1999). Clarithromycin may increase the rate of AZT absorption in AIDS patients, but this does not appear to have a significant impact on the overall bioavailability of AZT (Vance *et al.*, 1995). Another study (Polis *et al.*, 1997) demonstrated that simultaneous administration of AZT and clarithromycin appears to decrease the level of AZT in serum.

Overall, clarithromycin, like most of the macrolide antibiotics, exhibits fairly low acute toxicity. The acute oral LD_{50} is greater than 5 g/kg in ICR mice and CD rats and greater than 3 g/kg in Wistar rats. There was no difference in toxicity between juvenile and adult rats, although the LD_{50} values for weanling rats were considerably lower than for the older rats (1.2-1.3 g/kg). Treated weanlings showed reductions in suckling behavior and

spontaneous movement. No clarithromycin-related pathological lesions were seen. There was no apparent sex difference in sensitivity to clarithromycin at any age tested in acute studies (Craft and Siepman, 1993).

In several longer-term studies, the subchronic toxicity of clarithromycin was assessed in weanling and juvenile rats given daily oral doses for 2 to 6 weeks, with and without recovery periods. The dose in Wistar rats in a subchronic study at which no adverse effects occurred ranged from greater than 12.5 mg/kg per day to 55 mg/kg per day for juveniles and 8 to 50 mg/kg per day for adults. In the juvenile rats, most toxicity occurred at doses of 150 to 200 mg/kg per day, which is approximately 10 to 13 times the anticipated human dose (15 mg/kg per day). Toxic effects included dose-related decreases in feed consumption and body weight gain; erythema; salivation; alterations in a variety of hematologic and clinical chemistry parameters, including elevations in serum glucose and urine pH levels; and increases in liver and kidney weights. Mild vacuolar degeneration of the bile duct epithelium was apparent at doses of 150 to 800 mg/kg per day. The depressions in feed consumption and body weight gain normalized over the course of the studies, and most of the other adverse effects were reversible during recovery phases (Craft and Siepman, 1993; Guay *et al.*, 1993).

In beagles treated daily for up to 6 weeks with clarithromycin, the highest dose with no toxic effect was 100 mg/kg per day in juveniles and 4 to 100 mg/kg per day in adults (Craft and Siepman, 1993; Guay *et al.*, 1993). Vomiting occurred at doses higher than 30 mg/kg per day. At doses higher than 100 mg/kg per day, toxic effects included fatty deposition in both the liver and the kidney, cellular infiltration in the hepatic portal area, gastric glandular and thymic atrophy, cholecystitis, splenic lymphoid hyperplasia, anemia, ocular opacity, and increased liver, kidney, spleen, adrenal gland, and lung weights. Mortality was seen at doses of 100 to 400 mg/kg per day, and deaths at the higher doses were attributed to generalized organ degeneration. The authors reported no effect on cartilaginous growth plates in the younger animals.

In seven longer-term (unspecified duration) toxicity studies in monkeys (Guay *et al.*, 1993), the doses at which there were no toxic effects ranged from 25 to 100 mg/kg per day. Toxic effects observed in one or more studies at doses of 75 to 150 mg/kg per day usually normalized during the recovery period. When given orally, 150 mg/kg per day in monkeys is equivalent to approximately 2.4 times the recommended maximum human dose on a mg/m^2 basis (Abbott Laboratories, 1998). Toxic effects in monkeys consisted of reductions in feed consumption and body weight gain due to drug-induced vomiting, hypertransaminasemia, fatty changes in the liver, and increased liver, adrenal gland, and kidney weights. At higher doses (200 to 400 mg/kg per day), there were also lymphoid depletion, corneal opacity, fatty changes in renal cortical tubules, and histopathological changes in liver, thymus, stomach, and testes. At 100 mg/kg per day, death resulted from aspiration pneumonitis secondary to drug-induced

vomiting. Hepatic and renal damage occurred at 150 mg/kg per day and widespread parenchymal damage occurred in 90% of animals receiving 400 mg/kg per day.

Because of the renal toxicity seen in experimental animals at doses tenfold higher than the expected human dose of clarithromycin, tests were conducted in healthy human male volunteers to determine the renal toxicity. These volunteers received 500 mg clarithromycin every 12 hours for 13 doses, and their urinary levels of alanine aminopeptidase and N-acetyl-glucosaminidase were measured. Clarithromycin failed to produce an increase in either urinary enzyme, and the authors concluded that the drug is unlikely to produce significant nephrotoxicity at normal human doses (Chapelsky *et al.*, 1992).

The reproductive and developmental toxicity of clarithromycin has been evaluated in rats, mice, rabbits, and monkeys (Abbott Laboratories, 1998). Rats receiving daily doses of 150 to 160 mg/kg per day experienced no adverse effects on estrous cyclicity, fertility, parturition, or number or viability of pups, although the drug reached plasma levels twice those found in humans at the normal therapeutic dose. In primates given 150 mg/kg per day, plasma drug levels reached three times the human level and caused embryonic loss, although the fetotoxic effects of the drug were attributed to maternal toxicity accompanying the high dose rather than to direct fetotoxicity. Rabbits appear to be more sensitive to the reproductive toxicity of clarithromycin than other species tested. Pregnant rabbits receiving intravenous doses of 33 mg/m^2 (17 times lower than the maximum proposed human dose) suffered *in utero* fetal loss.

At least four teratogenicity studies of clarithromycin have been conducted in Wistar rats, three by the oral route and one by the intravenous route. The drug was administered during the period of major organogenesis at 160 mg/kg per day, and no teratogenic effects were observed. However, in other studies where Sprague-Dawley rats were given 150 mg/kg per day during gestational days 6 to 15, 2% to 6% of pups had cardiovascular or organ positional abnormalities (Guay *et al.*, 1993). Plasma drug levels in these rats reached twice human levels. In four studies conducted in mice, 500 to 1,000 mg/kg per day given during gestational days 6 to 15 produced a 3% to 30% incidence of cleft palates. Plasma drug levels in these mice were approximately 17 times those seen in humans after a normal therapeutic dose.

Two studies of the developmental toxicity of clarithromycin have been conducted in rabbits (at daily oral doses of up to 125 mg/kg and intravenous doses of 30 mg/kg administered during gestation days 6 to 18). As in the rats, no teratogenic effects were observed.

The reproductive and developmental toxicity of clarithromycin was tested in macaques given daily oral doses of 35 or 70 mg/kg. These doses produced no drug-related embryolethal, fetotoxic, or teratogenic effects, although 70 mg/kg did result in fetal growth retardation. However, there was significant maternal toxicity at 70 mg/kg, manifested mostly by vomiting, diarrhea, and decreased feed consumption and weight gain, and there was some question whether the observed growth retardation in the offspring was secondary to maternal toxicity. Plasma drug levels were twice human levels. No adequate and well-controlled reproductive toxicity studies have been conducted in pregnant women.

STUDY RATIONALE

Disseminated infection with MAC is one of the most common opportunistic infections in AIDS patients (Masur, 1993; Ong, 1999). AIDS patients with MAC receive combination therapy with an antiretroviral drug such as AZT and an antibiotic such as clarithromycin (CDC, 1997; Abbott Laboratories, 1998; Wright *et al.*, 1998), which is highly useful in treatment of disseminated MAC. Information on toxicity of AZT alone and clarithromycin alone is available. However, reproductive, developmental, and general toxic effects of AZT and clarithromycin combinations in animal models are not available. These reproductive, developmental, and general toxicity studies of the combination of AZT and clarithromycin in an animal model was conducted by the NIEHS as a part of the program to evaluate the toxicity of drugs, especially combination therapies used in the treatment of AIDS patients with opportunistic infections.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF CHEMICALS

3′-Azido-3′-deoxythymidine (AZT; Lots 7494-36-05 and 8404-120-03 RTI) was manufactured by Raylo Chemicals (Edmonton, Alberta) and supplied as a powder. Clarithromycin was manufactured by Abbott Laboratories as tablets that included excipients. The tablets for the first study were crushed and sieved to a powder (Lots 8405-10-03 RTI and 8406-110-02 RTI). Clarithromycin for the second study was extracted from the tablets using acetone and was supplied as a powder (Lot 8408-54-01 RTI). Identification of both compounds was confirmed by nuclear magnetic resonance and infrared spectroscopy. The purity of both AZT and the extracted clarithromycin was determined by high-performance liquid chromatography to be greater than 99%. The concentration of clarithromycin in human-dosage tablets was determined by high-performance liquid chromatography to be 55.3%.

The control article was an aqueous solution containing 0.5% methylcellulose. The methylcellulose (Lot 876672) was procured from Fisher Scientific Co. (Pittsburgh, PA).

DOSE FORMULATIONS

Each test article was mixed singly with the control article or in combination with the control article. The dose formulations were then mixed until visually homogeneous. Stability studies indicated that AZT/clarithromycin formulations in 0.5% methylcellulose were stable for at least 28 days when stored at approximately 5 ° C protected from light. The dose formulations used in these studies were stored refrigerated in the dark and were used within 27 days of mixing.

Samples of dose formulations were analyzed for concentration. Animal-room samples from formulations remaining after dosing were also analyzed for concentration. Results showed AZT concentrations of 98.0% to 108% and clarithromycin concentrations of 92.0% to 108% of the theoretical concentrations with one exception; the clarithromycin concentration in the 400 + 250 mg/kg formulation was 77.6% of the theoretical concentration. Animal-room samples from the same dose formulations were 95.3% to 113% of the theoretical concentrations for AZT and 99.2% to 105% of the theoretical concentrations for clarithromycin at all dose concentrations.

STUDY DESIGN

Swiss (CD-1®) mice were obtained from Charles River Laboratories, Portage, MI (Area P01), and were approximately 12 weeks old when placed on study. The mice were housed individually (males) or five per cage (females) during quarantine and were individually housed after randomization, except during cohabitation; they were housed in polycarbonate cages with solid bottoms and sides. All mice were uniquely identified by tail tattoo after randomization. The mice were housed in two rooms with equal numbers of each dose group and sex in each room. Average temperature in the animal rooms was 71.6° ± 1.40° F (SD) in Room 2 and 71.3° ± 1.29° F(SD) in Room 3; average relative humidity was 53.6% ± 3.37% (SD) in Room 2 and 51.2% ± 4.22% (SD) in Room 3.

Blood samples were collected from five sentinel animals per sex per room at terminal sacrifice as part of the animal disease screening program. Results indicated that all animals were free of viral antibodies.

AIDS patients are receiving combination therapy with clarithromycin and AZT. Controlled laboratory data are required to evaluate the potential toxicity of this combination therapy. At present, there are no adequate alternatives to whole animal models for this purpose. The Swiss mouse chosen for these studies is one of the mouse models routinely used for reproductive/developmental toxicity studies by the NIEHS.

The basic premise for dose selection is that the high-dose level should induce some measurable evidence of toxicity (e.g., anemia, weight loss, target organ toxicity). In a previous reproductive/developmental and toxicity study (NIEHS, 1999), AZT at the doses selected (200 and 400 mg/kg per day) caused resorptions and decreased litter sizes. Recent studies have also demonstrated dose-related decreases in hematologic parameters at these doses (NTP, 1999; NIEHS, 1999). The human therapeutic dose is 10 mg/kg per day (PDR, 1996). The selected doses were 20 and 40 times human doses, but on a body surface area basis, the doses were close to 2 and 4 times the therapeutic dose (Freireich, *et al.*, 1966).

According to the information provided in the manufacturer's insert (Abbott Laboratories package insert, 1998), the clinical dose of clarithromycin recommended for *Mycobacterium avium* complex (MAC) treatment in humans is 500 mg twice daily. Based on body surface area considerations, the dose levels of clarithromycin (250, 500, and 1,000 mg/kg per day) were approximately 1, 2, and 4 times the human therapeutic dose (Freireich, *et al.*, 1966).

The design of this study is a modification of a design published by Harris *et al.* (1992). A brief summary is provided in Table 1. The oral route of administration was selected because it is the route used in humans.

Combinations of AZT and clarithromycin were administered by gavage as a single formulation in an aqueous solution containing 0.5% methylcellulose. Concentrations of AZT in the formulations were 200 or 400 mg/kg body weight per day, and concentrations of clarithromycin were 250, 500, or 1,000 mg/kg day. Total daily doses of 20 mL/kg were divided into two equal doses of 10 mL/kg given approximately 6 hours apart. During the first study, due to the viscosity of the formulations, the concentrations were halved and the dose volume was increased to 40 mL/kg divided into two equal doses of 20 mL/kg given twice daily approximately 6 hours apart. Mice were divided into three groups as follows:

Male Mice

Ten males were assigned to each dose group, each of which was divided into group 1 and group 2. Prior to dosing, male mice were cohabited with female-B mice on study days 0 through 4. Male mice were dosed beginning on study day 5 through the day prior to sacrifice (except for those designated for plasma concentration level determinations). Males were cohabited with female-A mice on study days 9 through 13 to identify any effects of treatment on mating behavior. On study day 25 or 26, all male mice were weighed, and blood samples were obtained from the retroorbital sinus for hematology and clinical chemistry evaluations. The males were then euthanatized with CO_2, a gross necropsy was conducted, and the left testis and epididymis were collected and prepared for evaluation of sperm parameters as described in the Sperm Function Evaluation section. Eight additional male mice were assigned to selected dose groups to be used only for plasma AZT and clarithromycin concentration evaluations; these mice were treated on the same days as those assigned to the study, with the exception that they were dosed on the day of sacrifice. Body weight measurements for the extra males were used to calculate doses but were not reported.

Female-A Mice

Twenty females were assigned to each dose group, each of which was divided into group 1 and group 2. Female-A mice were dosed beginning on study day 0 through the day prior to sacrifice (except for those designated for plasma concentration level determination). Male mice were cohabited with female-A mice on study days 9 through 13 to identify any effects of treatment on mating behavior, fertilization, implantation, or the initial stages of development. During cohabitation periods, the females were examined daily for the presence of a vaginal plug as an indicator of pregnancy. If a vaginal plug was evident, that female was housed individually and that day was designated as day 0 of gestation. At the end of the cohabitation period, all animals were housed individually. Prior to parturition on day 18 of presumed gestation (study days 28 to 32), all female-A mice were weighed, and blood samples were taken from the retroorbital sinus for hematology and clinical chemistry evaluations. Female-A mice were then euthanatized with CO_2, and necropsy and caesarean section evaluations were conducted. Live fetuses

were removed, weighed, anesthetized on ice, and preserved in Bouin's fixative. The uterus of all sperm-negative females was examined for evidence of unsuccessful pregnancy, then press-plated between two heavy plates of glass to visualize implantation sites. Additional endpoints for all female-A mice included gravid uterine weight, number of implantation sites, resorptions, corpora lutea, and dead and live fetuses.

Female-B Mice

Twenty females were assigned to each dose group, each of which was divided into group 1 and group 2. Prior to dosing, the group B females were cohabited with males on study days 0 through 4. During the cohabitation period, the females were examined daily for the presence of a vaginal plug as an indicator of pregnancy. If a vaginal plug was evident, that female was housed individually and that day was designated as day 0 of gestation. At the end of the cohabitation period, sperm-negative female-B mice were euthanatized with CO_2 and discarded without necropsy; all other animals were housed individually. Sperm-positive female-B mice were assigned as evenly as possible across dose groups prior to gestation day 6. Female-B mice were subsequently dosed during gestation days 6 through 15, during the fetal organogenesis period, to identify effects on fetal development. Residual effects on parturition and the beginning of lactation were also evaluated. After gestation day 16, the bedding material and feeders were no longer changed. From gestation day 17 until the litters were delivered, female-B mice were observed twice daily for evidence of labor or delivery. The day delivery was completed was determined to the nearest day and was designated as postnatal day 0. On postnatal days 0 and 1, dam weights were recorded along with the number of live and dead pups, the number of male and female pups, gross malformations, and live pup weights. Dead pups were discarded. On postnatal day 4, female-B mice, including any that did not deliver, were weighed and blood samples were taken for hematology determinations. After the mice were euthanatized with CO_2, the uterus was removed and press-plated. All pups were weighed, given a thorough external examination for lesions or malformations, and the sex was recorded. The pups were then euthanatized with CO_2 and saved in Bouin's fixative.

Clinical Pathology

Blood was drawn at terminal sacrifice from all mice (except males designated for plasma drug levels determinations) for hematology determinations and from male and female-A mice for clinical chemistry determinations. All blood samples were taken from the retroorbital sinus under CO_2:O_2 (70:30) anesthesia and were collected into tubes containing EDTA (hematology) or no anticoagulant (clinical chemistry). Animals were selected in random order for blood collection and samples were analyzed in the order collected. Erythrocyte, platelet, and leukocyte counts; hematocrit; hemoglobin; mean cell hemoglobin; mean cell volume; mean cell hemoglobin concentration; leukocyte differentials; erythrocyte and platelet morphologies; and red cell distribution

width were determined on whole blood using a Technicon H·1™ automated hematology analyzer. Reticulocyte counts were conducted using a Coulter Model Elite™ flow cytometer. Blood smears were prepared to manually verify leukocyte differentials and morphologies if necessary, and platelet and erythrocyte morphological alterations were reported only in the raw data. Blood urea nitrogen, creatinine, alanine aminotransferase, total bile acids, alkaline phosphatase, and aspartate aminotransferase were determined based on sample availability using the Roche Cobas Fara™ automated analyzer.

Plasma AZT and Clarithromycin Levels

Blood was collected by retroorbital puncture into heparinized tubes at 30, 60, 120, and 240 minutes following the last dose and was used for determination of AZT and clarithromycin levels in plasma. Two male and two female-A mice per selected dose group were bled at each time point when possible; selected groups were those receiving 400 mg/kg AZT alone, clarithromycin alone at all dose levels, and clarithromycin at all dose levels in combination with 400 mg AZT. Eight extra males were added to each of these dose groups to be used only for plasma drug level determinations. Pregnant female-A mice were used, when possible. These female-A mice were caesarean-sectioned and necropsied 1 day earlier than remaining female-A mice in anticipation of mortality following blood collection.

The plasma samples were analyzed for AZT and its metabolites, 3′-azido-3′-deoxythymidine β-D-glucuronide (GAZT), 3′-amino-3′-deoxythymidine (AMT), and 3′-amino-3′-deoxythymidine glucuronide (GAMT), and for clarithromycin by high performance liquid chromatography.

Sperm Function Evaluation

The left testis from each male was removed at necropsy and weighed. The left epididymis was weighed, then the cauda epididymis was removed from the corpus. Sperm samples were collected from the cauda epididymis and mixed with modified Tyrode's Solution on two prewarmed slides per animal. Slides were maintained at approximately 37° C and were viewed under a light microscope by two separate observers to assess sperm motility. The distal cauda of the epididymis was weighed then placed in a petri dish containing phosphate-buffered saline, and the tissue was teased to release the contents. The final caudal epididymal sperm suspension was incubated for at least 15 minutes. An aliquot was then further diluted with saline solution and placed in a bath of hot water for at least one minute to kill the sperm. Sperm density was determined using a hemocytometer. To quantify spermatogenesis, testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemocytometer.

Necropsy and Histopathology

Necropsies were performed on all mice (except sperm-negative female-B mice) and histopathologic examiniations were conducted on the tissues listed in Table 1. All tissues were fixed in formalin, with the exception of the testis and epididymis, which were fixed in Bouin's. The tissues were cut (5-μm sections) and representative sections were mounted on glass microscope slides and stained with hematoxylin and eosin (testis and epididymis stained with PAS). All tissues were examined microscopically by a veterinary pathologist and results were described using standard nomenclature.

TABLE 1
Experimental Design and Materials and Methods for the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations in Swiss (CD-1®) Mice

Study Laboratory
Southern Research Institute, Birmingham, AL

Strain and Species
Swiss (CD-1®) mice

Animal Source
Charles River Laboratories, Portage, MI (Area P01)

Time Held Before Study
15 days

Average Age on Day 0 of Study
86 days

Date of Day 0
5/30/97 for group 1 mice and 6/6/97 for group 2 mice

Date of First Dose
Males day 5 (6/4/97 for group 1 and 6/11/97 for group 2 mice)
Females-A day 0 (5/30/97 for group 1 and 6/6/97 for group 2 mice)
Females-B gestation day 6 (6/6-10/97 for group 1 and 6/13-17/97 for group 2 mice)

Date of Last Dose
Males day prior to sacrifice (animals bled for plasma drug level determinations were dosed until the day of sacrifice)
Females-A day prior to sacrifice (animals bled for plasma drug level determinations were dosed until the day of sacrifice)
Females-B gestation day 15 (6/15-19/97 for group 1 and 6/22-26/97 for group 2 mice)

Days of Cohabitation
Males and Females-A days 9-13 (6/8-12/97 for group 1 and 6/15-19/97 for group 2)
Males and Females-B days 0-4 (5/30-6/3/97 for group 1 and 6/6-10/97 for group 2)
When possible, one male and two females within the same dosage group were housed together by consecutive animal number

Necropsy Dates
Males days 25-26 (6/24-25/97 for group 1 and 7/1-2/97 for group 2)
Females-A days 28-32 (gestation day 18, 6/27-7/1/97 for group 1 and 7/4-8/97 for group 2)
Females-B postnatal day 4 (6/23-27/97 for group 1 and 6/30-7/4/97 for group 2), these dates were also days 24-28 of presumed gestation for sperm-positive mice that did not deliver Sperm-negative mice were euthanatized on day 5 (6/4/97 for group 1 and 6/11/97 for group 2) after cohabitation

Average Age at Terminal Necropsy
Males 111-112 days
Females-A 114-118 days
Females-B 111-114 days

Size of Study Groups
10 or 18 males per dose group, 20 females-A per dose group, 20 females-B per dose group, each study group divided into group 1 and group 2

Method of Animal Distribution
Animals were assigned to groups using a stratified weight method and then assigned to study groups in random order

Method of Animal Identification
Tail tattoo

Diet
NIH-07 pelleted feed (Zeigler Bothers, Inc , Gardners, PA) available *ad libitum*, fresh feed provided weekly or as needed

TABLE 1
Experimental Design and Materials and Methods for the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations in Swiss (CD-1®) Mice

Water
Tap water (Birmingham municipal supply) via automatic watering system (Edstrom Industries, Inc , Waterford, WI) available *ad libitum*

Cages
Polycarbonate cages with solid bottom and sides (Lab Products, Inc , Maywood, NJ), changed once weekly, except during delivery and lactation periods (gestation day 16 to termination) for female-B mice

Bedding
Heat-treated hardwood bedding (P J Murphy Forest Products Corporation, Montville, NJ), changed once weekly, except during delivery and lactation periods (gestation day 16 to termination) for female-B mice

Cage Filters
Reemay spun-bonded polyester (Dupont, Wilmington, DE), changed once every 2 weeks

Racks
Stainless steel racks (Lab Products, Inc , Maywood, NJ), changed once every 2 weeks

Animal Room Environment
Mean temperature 71 6° ± 1 40° F in Room 2 and 71 3° ± 1 29°F in Room 3, mean relative humidity 53 6% ± 3 37% in Room 2 and 51 2% ± 4 22% in Room 3 Minimum of 10 air exchanges per hour Fluorescent light 12 hours per day

Doses
0 mg AZT + 0 mg clarithromycin per kg body weight per day
0 mg AZT + 250 mg clarithromycin per kg body weight per day
0 mg AZT + 500 mg clarithromycin per kg body weight per day
0 mg AZT + 1,000 mg clarithromycin per kg body weight per day
200 mg AZT + 0 mg clarithromycin per kg body weight per day
200 mg AZT + 250 mg clarithromycin per kg body weight per day
200 mg AZT + 500 mg clarithromycin per kg body weight per day
200 mg AZT + 1,000 mg clarithromycin per kg body weight per day
400 mg AZT + 0 mg clarithromycin per kg body weight per day
400 mg AZT + 250 mg clarithromycin per kg body weight per day
400 mg AZT + 500 mg clarithromycin per kg body weight per day
400 mg AZT + 1,000 mg clarithromycin per kg body weight per day

Type and Frequency of Observations
Mortality/morbundity twice daily
Clinical signs once daily
Vaginal plugs days 10-14 for females-A, days 1-5 for females-B
Body weights days 3, 5, 9, 13, 17, 21, 23, and sacrifice for males, days -1, 0, 4, 12, 16, 20, 23, 26, and sacrifice for females-A, gestation days 0, 8, 12, and 15 and postnatal days 0, 1, and 4 for females-B, postnatal days 0, 1, and 4 for F_1 pups

Clinical Pathology
Hematology and clinical chemistry evaluations were conducted on all males (except those designated for plasma AZT and clarithromycin level determinations) and females-A at terminal sacrifice, hematology evaluations were conducted on all females-B at terminal sacrifice

Plasma AZT and Clarithromycin Levels
Selected males and females-A from selected dose groups were bled for plasma AZT, AZT metabolites, and clarithromycin determinations

Sperm Function Evaluation
Conducted on all males at terminal sacrifice (except those designated for plasma AZT and clarithromycin level determinations)

TABLE 1
Experimental Design and Materials and Methods for the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations in Swiss (CD-1®) Mice

Necropsy and Histopathologic Examinations
Complete necropsies were performed on all breeder animals except sperm-negative females-B The right epididymis of males, the gravid uterus of females-A, and the liver of males and females-A were weighed at necropsy The following were collected at necropsy, saved in formalin, and examined histopathologically Males and females-A - brain, bone marrow (femur), gross lesions, heart, kidneys, liver, lungs and bronchi, mandibular and mesenteric lymph nodes, spleen, and thymus, also right testis and epididymis of males Females-B – gross lesions Pups were examined externally only and saved

NOTE The study design shown above is specific for the second study With the exception of clarithromycin dose levels (500, 1,250, and 2,500 mg/kg per day), the design of the initial study (conducted 11/8/96 - 12/17/96) was essentially the same

STATISTICAL METHODS

Paternal and maternal body weight evaluations were analyzed using Bartlett's Test of Homogeneity of Variances (Sokal and Rohlf, 1969) and the Analysis of Variance (Snedecor and Cochran, 1967a), when appropriate. If Bartlett's test was not significant ($P>0.05$) and the Analysis of Variance was significant ($P\leq0.05$), then Scheffe's test (1953) was used to identify the statistical significance of individual groups. If Bartlett's test was significant ($P\leq0.05$), then the Analysis of Variance was not appropriate and the Kruskal-Wallis test (Sokal and Rohlf, 1969) was used; in cases where the Kruskal-Wallis test was significant ($P\leq0.05$), Dunn's (1964) method of multiple comparisons was used to identify the statistical significance of individual groups. These methods were also used to analyze fetal body weight and pup body weight (per litter), as well as all other evaluations involving continuous data. Observations for delivered and dead conceptuses of the female-A dams were excluded from fetal body weight summaries and statistical analyses.

Group means and standard deviations were calculated for hematology and clinical chemistry parameters and for terminal body and epididymis weights. Liver/body weight ratios were also calculated. Mean terminal body weights, mean liver weights, and liver/body weight ratios for each treated group were compared to those of the control group by a two-tailed Student's t-test for each sex. The standard deviations used in the t-tests were obtained by pooling the individual values for the control and treated groups. Hematology and clinical chemistry data were evaluated using Dunnett's (1955) test.

Sperm end points (i.e., terminal body, epididymis, cauda epididymis, and testis weights, sperm motility, and sperm concentration) were analyzed using a two-way analysis of variance. Statistical significance in the absence of significant interaction between AZT and clarithromycin was indicative of a treatment-related effect. If the interaction was not statistically significant, control and treated group means were compared using a multiple

comparison procedure; Williams' test (1971, 1972) was applied if there was an indication of trend ($P<0.01$), and Dunnett's test (1955) was used in the absence of a trend, as assessed by Jonckheere's test (1954).

Proportion data (e.g., clinical observation data and the incidences of pregnancy, resorption, death, and total resorption) for presumed pregnant mice were analyzed using the Cochran-Armitage test for a linear trend in proportions (Snedecor and Cochran, 1967b) and Fisher's exact test (Siegel, 1956).

RESULTS

In the initial study with AZT (200 or 400 mg/kg per day) and clarithromycin (500, 1,250, or 2,500 mg/kg per day), the dose levels of clarithromycin were too high, resulting in excessive mortality. Clarithromycin dose levels were approximately 2, 5, and 10 times the human therapeutic dose, and 100% mortality occurred in male and female-A groups treated with 2,500 mg/kg clarithromycin alone or in combination with AZT. The mortality was attributed to toxicity in multiple vital organs and localized complications in the stomach associated with chronic dilatation from the large volume of thick gavaged material. The unpurified clarithromycin formulations contained numerous excipients included by the manufacturer in the preparation of these antibiotic tablets for human therapeutic use. The excipients likely contributed to the viscosity of the clarithromycin dosage formulations, resulting in chronic dilatation of the stomach. The local antibacterial action of clarithromycin apparently altered the normal flora in the stomach lumen, resulting in a fungal overgrowth and inflammatory lesions. Other lesions attributed to clarithromycin occurred in the bone marrow, spleen, liver, kidney, heart, brain, thymus, lymph nodes, and salivary gland. Extensive vacuolization of phagocytic cells occurred in the majority of these tissues. Groups treated with AZT and clarithromycin combinations developed severe hematopoietic toxicity with bone marrow depression, anemia, and high mortality. Reduced fertility also occurred in groups treated with higher dosages of clarithromycin alone or in combination with AZT. With the exception of Plates illustrating microscopic lesions caused by clarithromycin at 1,250 mg/kg (considered comparable to the 1,000 mg/kg administered in the second study), the initial study will not be referred to again in this report. The remainder of this report deals with results in Swiss CD-1® mice treated by oral gavage with 200 or 400 mg/kg AZT and 250, 500, or 1,000 mg/kg clarithromycin.

SURVIVAL AND CLINICAL FINDINGS

Male Mice

Administration of 200 or 400 mg/kg AZT alone or 250, 500, or 1,000 mg/kg clarithromycin alone to male mice for approximately 20 days did not result in mortality. No deaths occurred in any of the male groups treated with combinations of AZT and clarithromycin.

Piloerection, believed to be a nonspecific manifestation of poor health, was the most common clinical finding. Piloerection occurred in 5 and 10 male mice, respectively, treated with 200 and 400 mg/kg AZT alone. Treatment with 500 and 1,000 mg/kg clarithromycin alone resulted in piloerection in 9 and 10 males, respectively. For the

male groups treated with 200 mg/kg AZT plus 250, 500, or 1,000 mg/kg clarithromycin, the respective incidences of piloerection were 3, 5, and 10. Piloerection occurred in all 10 males in each of the groups treated with 400 mg/kg AZT in combination with 250, 500, or 1,000 mg/kg clarithromycin. Pallor occurred in three male mice treated with 200 mg/kg AZT plus 1,000 mg/kg of clarithromycin and in one and seven males, respectively, treated with 400 mg/kg AZT plus 500 or 1,000 mg/kg clarithromycin. The pallor was believed to be a reflection of the anemia evident in the hematology parameters. An ulcer was seen in the groin area of one male treated with 400 mg/kg AZT plus 1,000 mg/kg clarithromycin.

Female-A Mice

Three female-A mice, treated for approximately 30 days, died prior to scheduled sacrifice. One female-A mouse each in the groups treated with 1,000 mg/kg clarithromycin alone, 400 mg/kg AZT plus 500 mg/kg clarithromycin, and 400 mg/kg AZT plus 1,000 mg/kg clarithromycin was sacrificed due to moribund condition. All three mice had piloerection and/or pallor. The female treated with 1,000 mg/kg clarithromycin alone also had sunken eyes and was cold to the touch. Labored breathing, abdominal distention, cold to the touch, and sunken eyes were other clinical findings in the female treated with 400 mg/kg AZT plus 500 mg/kg clarithromycin that died early. Gavage accidents did not occur.

With the exception of piloerection and pallor, most adverse clinical findings occurred in the three mice that were sacrificed moribund prior to scheduled termination. As with the male mice, piloerection was the most common clinical finding. Piloerection occurred in one female-A mouse treated with 400 mg/kg AZT alone and 19 female-A mice treated with 1,000 mg/kg clarithromycin alone. For the groups treated with 200 mg/kg AZT plus 250, 500, or 1,000 mg/kg clarithromycin, the respective incidences of piloerection were 1, 2, and 20. For the female-A groups treated with 400 mg/kg AZT plus 250, 500, or 1,000 mg/kg clarithromycin, the incidences of piloerection were 7, 20, and 20. Pallor occurred in two female-A mice treated with 1,000 mg/kg clarithromycin alone. Pallor did not occur in groups treated with AZT alone. The incidence of pallor generally increased as combination dosages of AZT and clarithromycin increased. The respective incidences of pallor in female-A groups treated with 200 mg/kg AZT in combination with 250, 500, or 1,000 mg/kg clarithromycin were one, zero, and three. For the groups that received 400 mg/kg AZT in combination with 250, 500, or 1,000 mg/kg clarithromycin, the incidences were 13, 19, and 20, respectively. The occurrence of pallor was believed to be a reflection of the anemia evident in the hematology parameters. Other clinical observations documented with a low incidence were sunken eyes, cold to the touch, abdominal distention, and labored breathing.

Female-B Mice

A total of five female-B mice, treated for approximately 10 days, died prior to scheduled sacrifice. One female-B mouse each died or was sacrificed moribund in the groups treated with 500 mg/kg clarithromycin alone, 200 mg/kg AZT plus 500 mg/kg clarithromycin, 400 mg/kg AZT plus 250 mg/kg clarithromycin, and 400 mg/kg AZT plus 500 mg/kg clarithromycin, and one female-B mouse in the control group was sacrificed in a moribund condition. None of these deaths were gavage accidents.

As with the female-A group, the majority of the clinical findings, other than piloerection and pallor, occurred in female-B mice that died prior to scheduled sacrifice. During the gestation period, piloerection occurred in 11 female-B mice treated with 200 mg/kg AZT plus 1,000 mg/kg clarithromycin and in one, nine, and eight female-B mice, respectively, treated with 400 mg/kg AZT plus 250, 500, or 1,000 mg/kg clarithromycin. During the lactation period, piloerection occurred in seven mice treated with 200 mg/kg AZT plus 1,000 mg/kg clarithromycin and three treated 400 mg/kg AZT plus 500 mg/kg clarithromycin. During the gestation period, pallor, believed to be a reflection of anemia, occurred in seven, eight, and seven female-B mice treated with 400 mg/kg AZT plus 250, 500, or 1,000 mg/kg clarithromycin, respectively. During the lactation period, pallor occurred in one, three, and one females treated with 200 mg/kg AZT plus 1,000 mg/kg clarithromycin and with 400 mg/kg AZT plus 250 or 500 mg/kg clarithromycin, respectively. Other clinical findings occurring with low incidences were sunken eyes, decreased motor activity, cold to the touch, and labored breathing. These findings were not considered related to either test article because most were single nondosage-dependent events.

BODY WEIGHTS AND ORGAN WEIGHTS

Male Mice

Administration of 200 or 400 mg/kg AZT alone or 250, 500, or 1,000 mg/kg clarithromycin alone did not affect body weight or body weight gains (Figure 1 and Table A1). Administration of 200 or 400 mg/kg AZT in combination with 250, 500, or 1,000 mg/kg clarithromycin had no effect on body weight or body weight gains. The slight significant ($P \leq 0.05$) elevation in terminal body weights of male mice (Table A2) treated with 400 mg/kg AZT plus 250 mg/kg clarithromycin was not considered to be biologically significant as the elevation was minor, and a dose-related pattern was not evident.

A significant elevation ($P \leq 0.05$) in the mean absolute liver weight (Figure 4 and Table A2) occurred in males treated with 1,000 mg/kg clarithromycin alone. This minor elevation was not considered to be biologically significant, as a dose-related pattern did not occur, and corresponding elevations in clinical chemistry parameters

indicative of liver toxicity did not occur. Treatment with 200 or 400 mg/kg AZT alone or in combination with 250, 500, or 1,000 mg/kg clarithromycin had no impact on liver weights of male mice.

Female-A Mice

Reduced body weight gains (Figure 2 and Table A1) occurred in the female-A groups treated with 200 ($P \leq 0.05$) or 400 ($P \leq 0.01$) mg/kg AZT alone, 1,000 mg/kg clarithromycin alone, 200 mg/kg AZT in combination with 250, 500 ($P \leq 0.01$), or 1,000 ($P \leq 0.01$) mg/kg clarithromycin, and 400 mg/kg AZT in combination with 250, 500, or 1,000 mg/kg clarithromycin ($P \leq 0.01$ in all groups). In general, the reduced body weights corresponded with reduced gravid uterine weights as significant declines ($P \leq 0.05$) in mean corrected body weights (terminal body weight minus gravid uterine weight) did not occur.

Significant declines ($P \leq 0.05$) in absolute liver weights occurred in female-A groups treated with 400 mg/kg AZT alone and in the groups treated with 400 mg/kg AZT in combination with 250 or 500 mg/kg clarithromycin (Figure 4 and Table A2). Relative liver weights, which take body weights into consideration, were lower ($P \leq 0.05$) in the groups treated with 400 mg/kg AZT alone and 400 mg/kg AZT plus 500 mg/kg clarithromycin. These slight declines in liver weights were not considered to be a reflection of liver toxicity but tended to parallel slight declines in corresponding body weights.

Female-B Mice

Slight reductions in average body weight gains and body weights occurred in female-B groups treated with 200 mg/kg AZT in combination with 500 or 1,000 mg/kg clarithromycin and in the groups treated with 400 mg/kg AZT plus 250, 500, or 1,000 mg/kg clarithromycin (Figure 3 and Table A1). The decline in average body weight was significant ($P \leq 0.05$) in the group treated with 400 mg/kg AZT plus 1,000 mg/kg clarithromycin. A similar pattern of reduced body weight gains occurred during gestation and lactation periods. In general, the more severely affected groups also had reductions in live litter size or no live litters (400 mg/kg AZT plus 1,000 mg/kg clarithromycin).

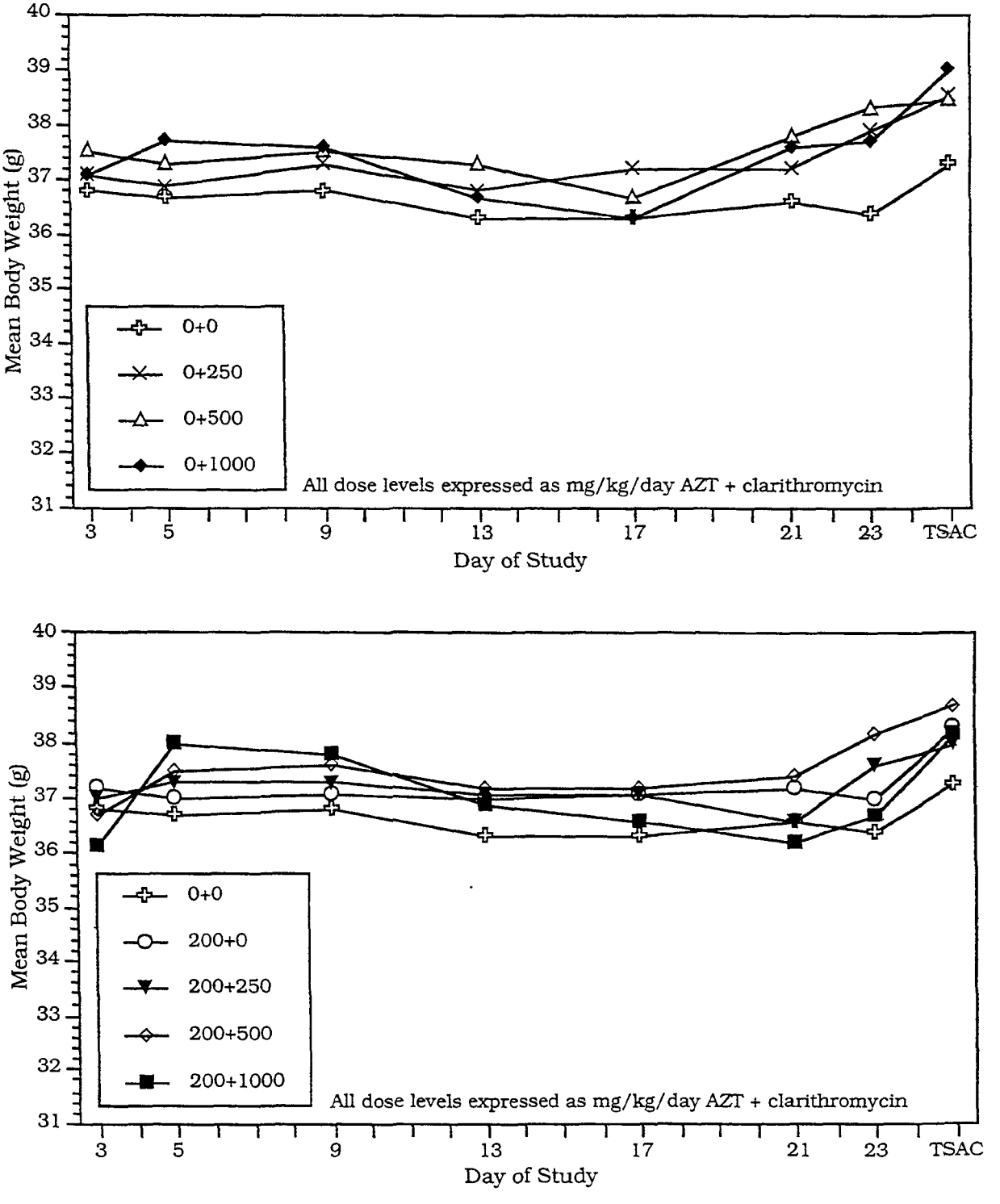


FIGURE 1
Mean Body Weights of Male Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations
(TSAC = Terminal Sacrifice)

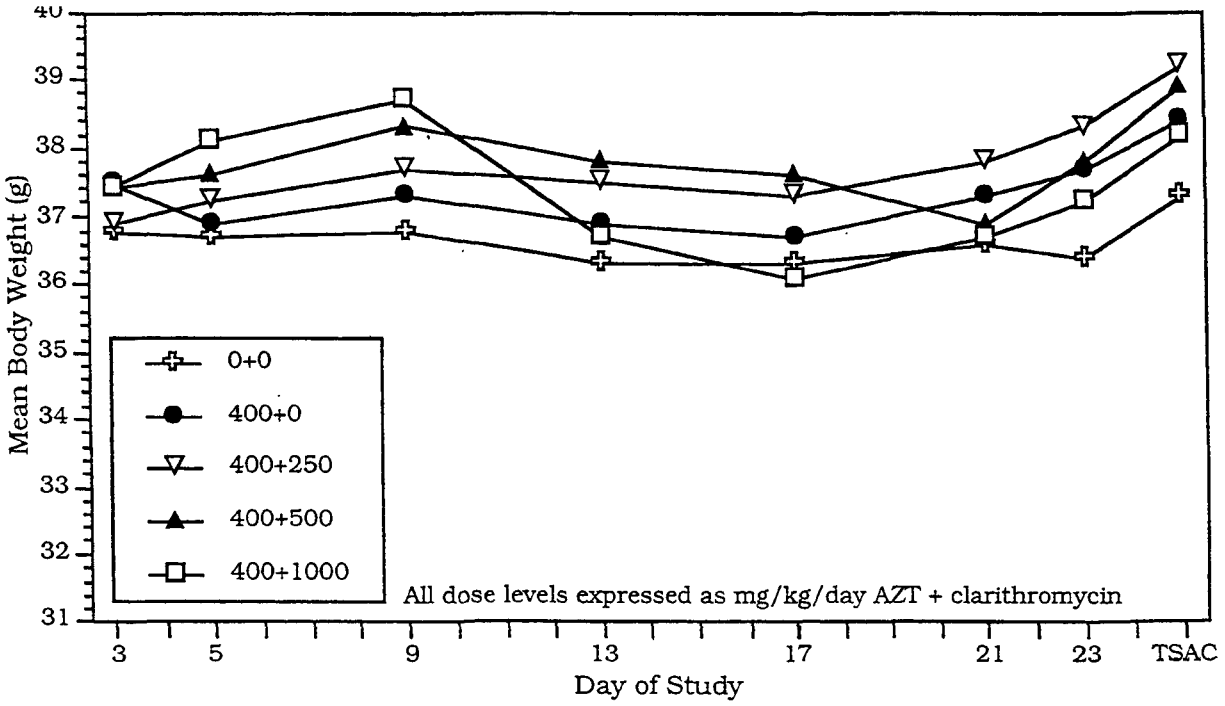


FIGURE 1 (continued)
Mean Body Weights of Male Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations
(TSAC = Terminal Sacrifice)

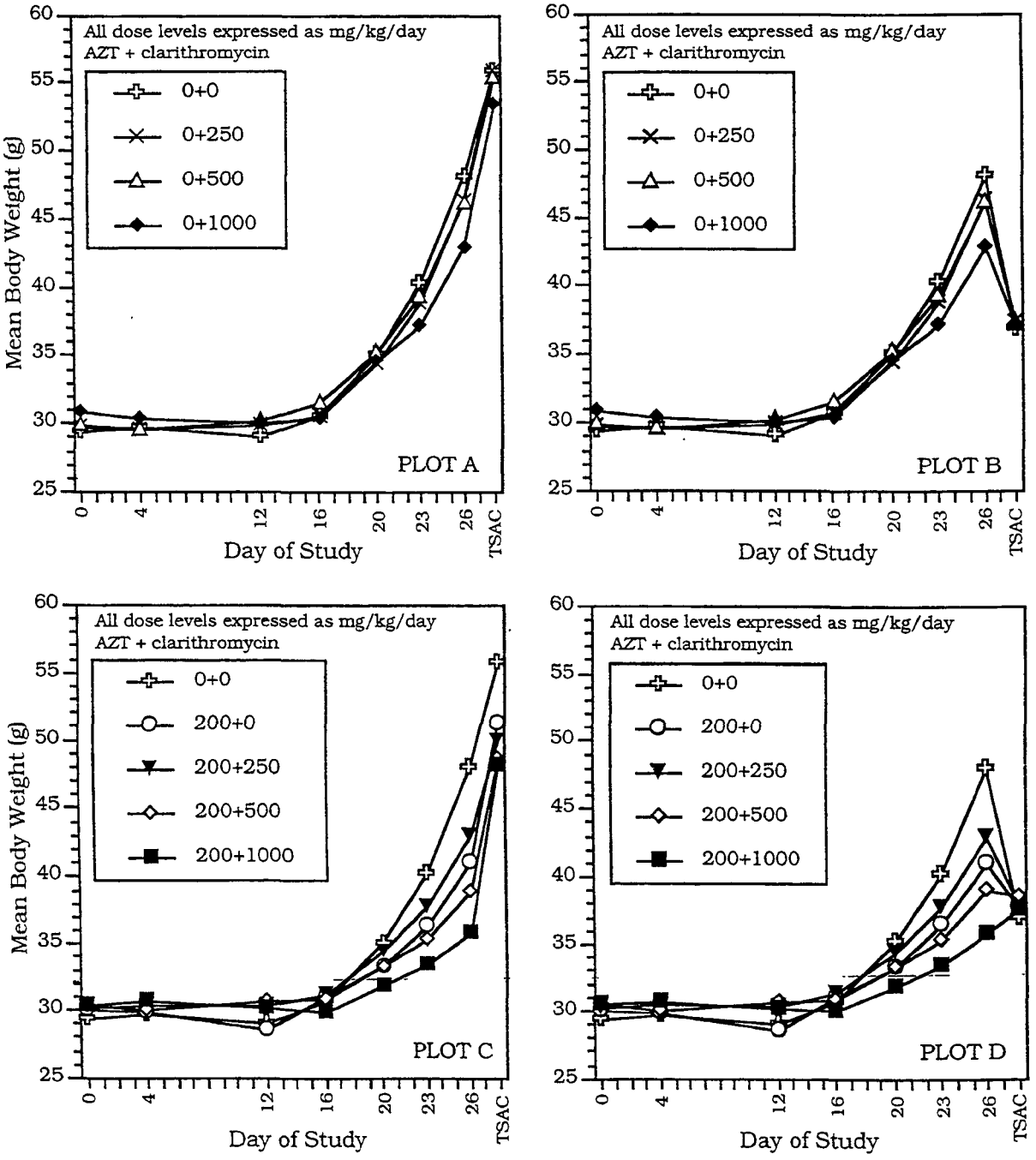


FIGURE 2
Mean Body Weights of Female-A Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations
[Plots A, C, and E show body weights from day 0 through terminal sacrifice (TSAC). Plots B, D, and F show the identical data except body weights at TSAC are after gravid uterine weights have been subtracted. Group mean body weights include only values for dams that were actually pregnant and were sacrificed and caesarean-sectioned as scheduled on presumed day 19 of gestation.]

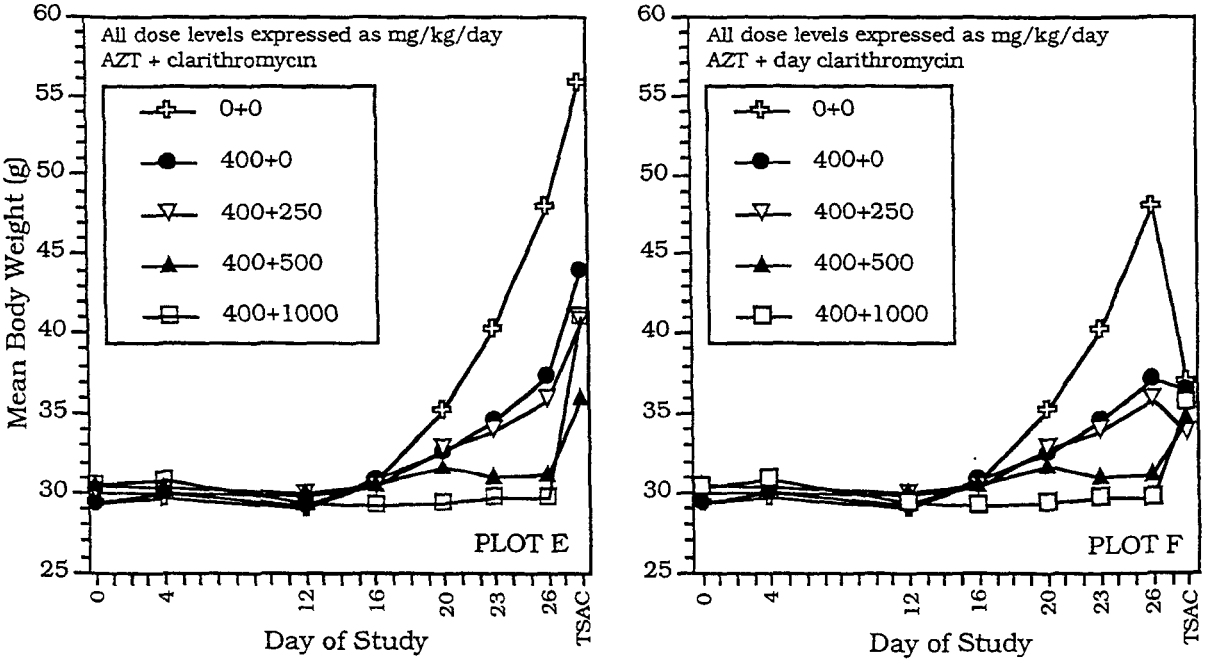


FIGURE 2 (continued)

Mean Body Weights of Female-A Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations

[Plots A, C, and E show body weights from day 0 through terminal sacrifice (TSAC). Plots B, D, and F show the identical data except body weights at TSAC are after gravid uterine weights have been subtracted. Group mean body weights include only values for dams that were actually pregnant and were sacrificed and caesarean-sectioned as scheduled on presumed day 19 of gestation.]

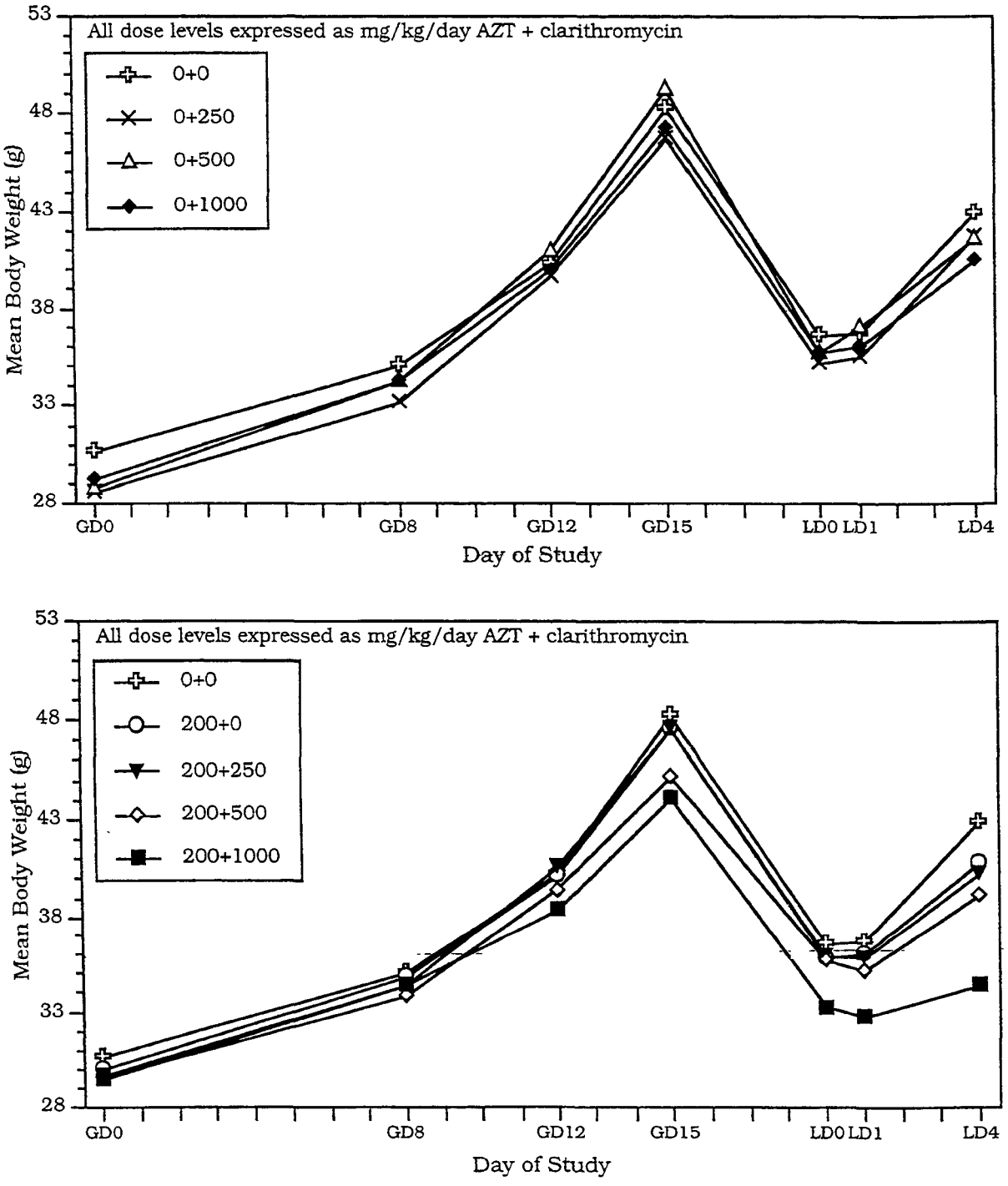


FIGURE 3
Mean Body Weights of Female-B Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations
(GD = Gestation Day; LD = Lactation Day. Gestation period body weights include only dams that were actually pregnant; lactation period body weights include only dams that delivered litters and had surviving pups.)

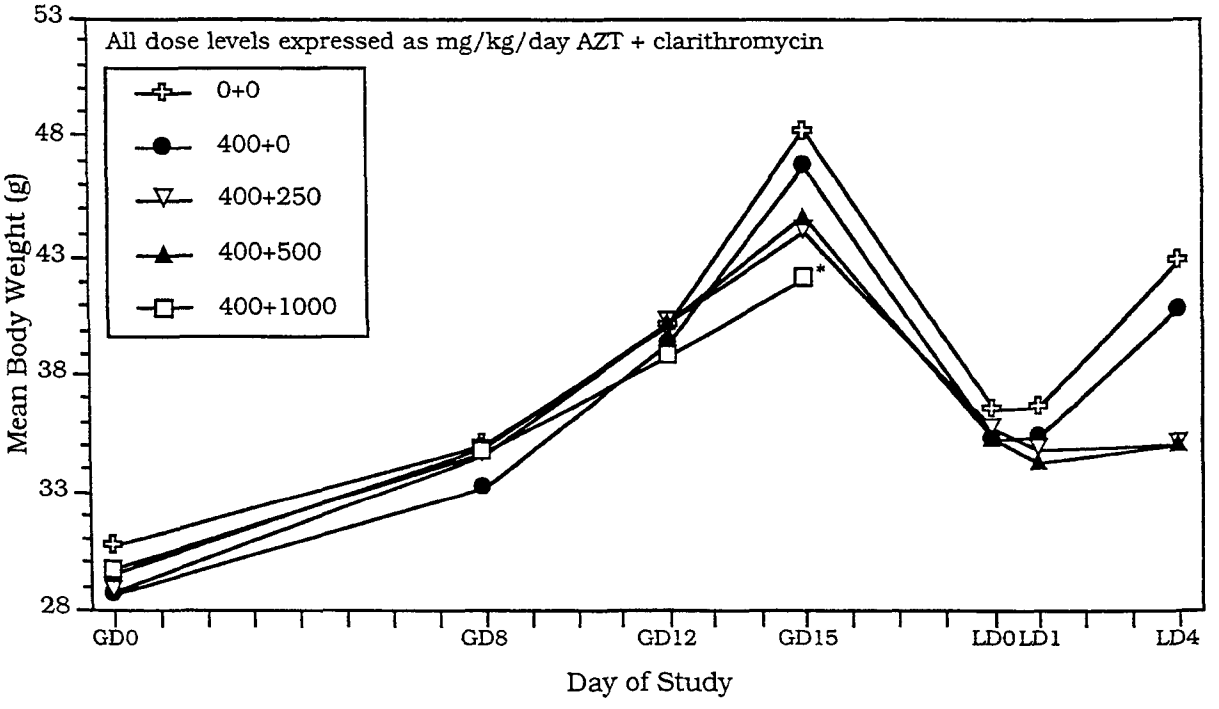


FIGURE 3 (continued)

Mean Body Weights of Female-B Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations

(GD = Gestation Day; LD = Lactation Day, *no litters were delivered in the 400 + 1,000 mg/kg group. Gestation period body weights include only dams that were actually pregnant; lactation period body weights include only dams that delivered litters and had surviving pups.)

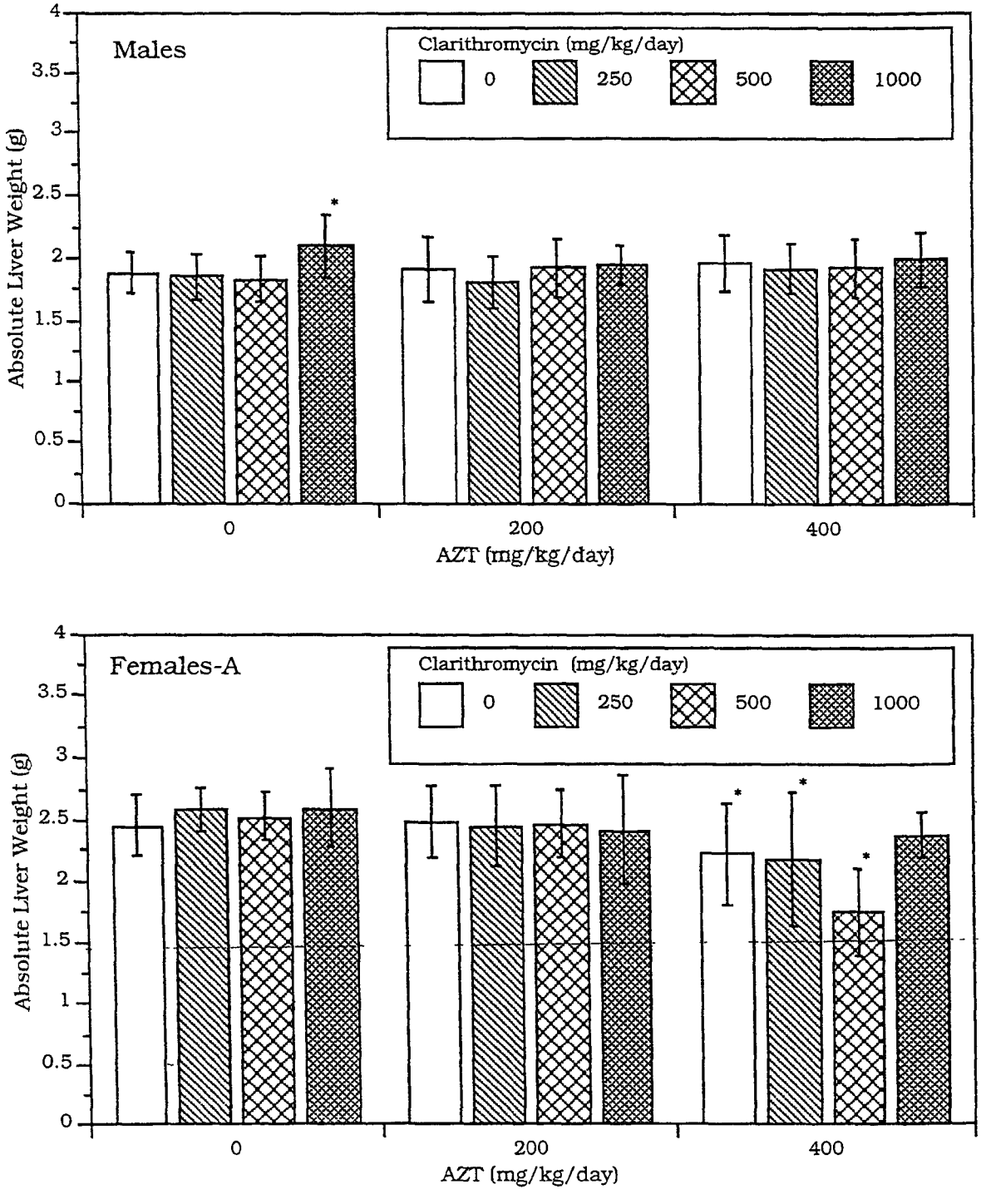


FIGURE 4
Mean Absolute Liver Weights for Male and Female-A Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations
[Bars indicate standard deviation, * = significantly different ($P<0.05$) from control group using the Student's *t*-test.]

CLINICAL PATHOLOGY

Hematology

AZT Alone

Administration of AZT alone caused mild declines in red blood cell (RBC), hemoglobin (Hgb), and hematocrit (Hct) values and mild to moderate increases in mean cell volume (MCV) and red cell distribution width (RDW) in both male and female mice. Mean RBC counts for mice (Figure 5 and Table B1) treated with 200 or 400 mg/kg AZT were, respectively, 10% (9.29 × 10^6/μL) and 23% (7.95 × 10^6/μL, P<0.01) lower in males, and 11% (7.60 × 10^6/μL) and 10% (7.75 × 10^6/μL) lower in female-A mice than the mean RBC counts in the respective control groups (10.34 and 8.57 × 10^6/μL). In general, declines in Hgb and Hct values (Table B1) paralleled the declines in RBC counts. For female-B mice treated for approximately 10 days, slight declines in mean RBC counts occurred (Table B1); however, the changes were too minimal to be considered biologically significant.

The declines in RBC counts in male and female-A mice treated with AZT alone were accompanied by mild to moderate macrocytosis and increased RDW values, changes indicative of increased anisocytosis of erythrocytes. In general, the macrocytosis was most evident in female-A mice treated for approximately 30 days, slightly less severe in males treated for approximately 20 days, and least evident in female-B mice treated for approximately 10 days. MCV values for female-A mice (Figure 6 and Table B1) treated with 200 or 400 mg/kg AZT were both approximately 13% (55.5 fL and 55.3 fL, P<0.01) greater than that in the control group (49.0 fL). In male mice treated with the same dosages, MCV values were approximately 8% (50.3 fL, P<0.05) and 13% (52.4 fL, P<0.01) greater than that (46.5 fL) in the control group. Female-B mice had respective MCV values approximately 5% (54.4 fL, P<0.01) and 6% (54.7 fL, P<0.01) greater than that (51.6 fL) in the control group. Elevations in mean cell hemoglobin (MCH) values paralleled the elevations in MCV values in both sexes. Reticulocytosis did not accompany the macrocytosis in any of the AZT treatment groups.

A treatment-related increase in RDW values, indicative of anisocytosis, occurred and, in general, males and female-A mice had the greatest increases and female-B mice had the smallest increases. Respective mean RDW values for female-A mice (Figure 7 and Table B1) treated with 200 or 400 mg/kg AZT were approximately 1.2 fold (19.9%) and 1.3 fold (21.1%, P<0.05) greater than the mean (16.0%) in the controls. Male mice treated with identical dosages had mean RDW values approximately 1.4 fold (19.8%, P<0.01) and 1.6 fold (22.3%, P<0.01) greater than the control group mean (13.9%). Respective mean RDW values for female-B mice (Table B1) treated with 200 and 400 mg/kg AZT were approximately 1.2 fold (19.9%, P<0.01) and 1.3 fold (21.4%, P<0.01) greater than the control group mean (17.1%).

No consistent treatment-related changes occurred in leukocyte or platelet parameters subsequent to administration of 200 or 400 mg/kg AZT (Table B1). The slight, but minimal, lymphocytosis ($5.21 \times 10^3/\mu L$, $P<0.05$) in female-A mice treated with 400 mg/kg AZT was not considered treatment related because the response was not dose related.

The dysplastic changes in erythrocytes (elevated RDW and MCV values in the absence of reticulocytosis) that accompanied the slight treatment-related anemia indicate that mild treatment-related erythropoietic toxicity occurred. The slight nonregenerative macrocytic anemia is compatible with results reported in $B6C3F_1$ mice (Thompson, *et al.*, 1991). The greater effects in female-A mice likely reflect the longer duration of treatment (approximately 30 days) when compared to males (approximately 20 days) and females-B (approximately 10 days). The alterations in erythrocytes indicate that increased RBC turnover occurred, which is compatible with the increased hematopoietic cell proliferation and hemosiderosis found microscopically in the spleens of male and female-A mice.

Clarithromycin Alone

Administration of 250, 500, or 1,000 mg/kg clarithromycin alone to male and female mice did not result in consistent changes in erythrocyte parameters (Table B1). A leukocytosis occurred in the female-A mice treated with 1,000 mg/kg clarithromycin; the mean WBC count was approximately 2.2 fold ($12.74 \times 10^3/\mu L$, $P<0.01$) greater than the mean in the control group ($5.92 \times 10^3/\mu L$). Evaluation of the differential data revealed significant increases in neutrophils (Figure 10 and Table B1) and lymphocytes, with respective values approximately 3.3 fold ($5.71 \times 10^3/\mu L$, $P<0.01$) and 1.5 fold ($5.89 \times 10^3/\mu L$, $P<0.01$) greater than those of the control group (1.71 and $3.92 \times 10^3/\mu L$). Significant alterations in leukocyte counts did not occur in male or female-B mice treated with clarithromycin alone (Table B1).

AZT and Clarithromycin Combinations

AZT in combination with clarithromycin resulted in a treatment-related anemia of far greater severity than the anemia that occurred with AZT alone. In general, the severity of the hematological changes was greatest in female-A mice treated for approximately 30 days, less in male mice treated for approximately 20 days, and least prominent in female-B mice treated for approximately 10 days. Combination therapy also caused macrocytosis and microcytosis, increases in RDW values, reticulocytopenia, leukocytosis and leukopenia, neutrophilia, lymphopenia, and thrombocytosis.

Respective mean RBC counts in female-A mice treated with 200 mg/kg AZT plus 250, 500, or 1,000 mg/kg clarithromycin (Figure 5 and Table B1) were approximately 23% ($6.59 \times 10^6/\mu L$, $P<0.01$), 22% ($6.66 \times 10^6/\mu L$, $P<0.01$), and 30% ($6.01 \times 10^6/\mu L$, $P<0.01$) lower than the mean ($8.57 \times 10^6/\mu L$) in the control group. For the female-A groups treated with 400 mg/kg AZT plus the same dosages of clarithromycin, respective mean RBC counts were approximately 39% ($5.22 \times 10^6/\mu L$, $P<0.01$), 71% ($2.45 \times 10^6/\mu L$, $P<0.01$), and 57% ($3.66 \times 10^6/\mu L$, $P<0.01$) lower than the control mean ($8.57 \times 10^6/\mu L$). The anemia was less severe in male groups treated with AZT and clarithromycin combinations for approximately 20 days than in female-A groups treated for approximately 30 days. Respective mean RBC counts in males treated with 400 mg/kg AZT plus 250, 500, or 1,000 mg/kg clarithromycin were approximately 33% ($6.94 \times 10^6/\mu L$, $P<0.01$), 32% ($7.02 \times 10^6/\mu L$, $P<0.01$), and 42% ($5.95 \times 10^6/\mu L$, $P<0.01$) lower than the control mean ($10.34 \times 10^6/\mu L$). In general, Hgb and Hct values paralleled the changes in RBC counts. Significant ($P<0.05$) alterations in RBC, Hgb, and Hct values did not occur in female-B mice receiving combination therapy for approximately 10 days.

In general, the anemia that occurred with combination therapy was accompanied by erythrocytic macrocytosis in lower-dose combination groups and reversal of this change toward normocytic or microcytic anemia at higher dose combination levels. Respective MCV values for female-A mice (Figure 6 and Table B1) treated with 200 mg/kg AZT plus 250, 500, or 1,000 mg/kg clarithromycin were approximately 18% (57.6 fL, $P<0.01$), 22% (59.7 fL, $P<0.01$), and 8% (53.0 fL) greater than the mean (49.0 fL) in the control group. Female-A mice treated with 400 mg/kg AZT plus the same dosages of clarithromycin had respective MCV values approximately 9% higher (53.6 fL, $P<0.05$), 9% lower (44.5 fL), and 4% lower (46.8 fL) than the control mean. Respective MCV values for males treated with 200 mg/kg AZT plus 250, 500, or 1,000 mg/kg clarithromycin were approximately 10% (51.0 fL, $P<0.01$), 9% (50.6 fL, $P<0.01$), and 5% (48.9 fL) higher than the control mean (46.5 fL). Males treated with 400 mg/kg AZT plus the same dosages of clarithromycin had respective MCV values approximately 9% (50.6 fL, $P<0.05$), 7% (49.7 fL), and 2% (47.5 fL) higher than the control mean. In female-B groups treated with combinations for approximately 10 days, MCV values ranged from 4% (53.7 fL) for the 400 mg/kg AZT plus 250 mg/kg clarithromycin group to 7% (55.4 fL, $P<0.01$) for the with 200 mg/kg AZT plus 500 mg/kg clarithromycin group, greater than the mean (51.6 fL) in the controls. In general, the calculated MCH values tended to parallel MCV values in all dosage groups for both sexes.

Anisocytosis, measured by increased RDW values, occurred in males and females given combination therapy. As with MCV values, greater increases in RDW values tended to occur in groups treated with lower dose combinations. Respective mean RDW values in female-A groups (Figure 7 and Table B1) treated with 200 mg/kg AZT plus 250, 500, or 1,000 mg/kg clarithromycin were approximately 1.6 fold (25.8%, $P<0.01$), 1.6 fold (26.7%,

P<0.01), and 1.5 fold (24.6%, P<0.01) greater than the mean (16.0 %) in the control group. The mean RDW value in the female-A group treated with 400 mg/kg AZT plus 250 mg/kg clarithromycin was approximately 1.6 fold (25.4%, P<0.01) greater than the control mean. Significant alterations (P<0.05) in RDW values did not occur in female-A groups treated with 400 mg/kg AZT plus 500 or 1,000 mg/kg clarithromycin. Respective mean RDW values for males treated with 200 mg/kg AZT plus 250, 500, or 1,000 mg/kg clarithromycin were approximately 1.6 fold (21.7%, P<0.01), 1.7 fold (23.1%, P<0.01), and 1.5 fold (21.5%, P<0.01) greater than the control mean (13.9%). For males treated with 400 mg/kg AZT plus the same dosages of clarithromycin, respective mean RDW values were approximately 1.7 fold (24.2%, P<0.01), 1.7 fold (24.2%, P<0.01), and 1.6 fold (21.7%, P<0.01) greater than the control mean. Milder elevations in RDW values occurred in all female-B groups treated with combination therapy, with values 1.3 to 1.4 fold (22.0%, P<0.01, to 23.9%, P<0.01) greater than the mean (17.1%) in the control group.

Reticulocytopenia accompanied the treatment-related anemia in the higher-dose female-A combination groups. Respective mean reticulocyte counts in female-A groups treated with 400 mg/kg AZT plus 500 or 1,000 mg/kg clarithromycin (Figure 8 and Table B1) were approximately 85% ($0.6 \times 10^5/\mu L$, P<0.01) and 51% ($1.9 \times 10^5/\mu L$) lower than the mean ($3.9 \times 10^5/\mu L$) in the control group. Significant alterations (P<0.05) in reticulocyte counts did not occur in male or female-B mice. There was no instance in which significant elevations (P<0.05) in reticulocyte counts accompanied the significant (P<0.05) macrocytosis or increased RDW values.

Thrombocytosis occurred in male and female-A mice treated with combination therapy. Respective mean platelet counts for female-A mice (Figure 9 and Table B1) treated with 200 mg/kg AZT plus 250, 500, or 1,000 mg/kg clarithromycin were approximately 1.4 fold ($1,442 \times 10^3/\mu L$, P<0.01), 1.3 fold ($1,332 \times 10^3/\mu L$), and 1.3 fold ($1,386 \times 10^3/\mu L$, P<0.05) greater than the mean ($1,034 \times 10^3/\mu L$) in the control group. For the female-A groups treated with 400 mg/kg AZT and 250, 500, or 1,000 mg/kg clarithromycin, respective mean platelet counts were approximately 1.7 fold ($1,750 \times 10^3/\mu L$, P<0.01), 1.9 fold ($1,982 \times 10^3/\mu L$, P<0.01), and 1.6 fold ($1,645 \times 10^3/\mu L$, P<0.01) higher than the control mean. Although not statistically significant, platelet counts in male mice treated with combination therapy ranged from 1.3 fold ($1,095 \times 10^3/\mu L$) in the 200 mg/kg AZT plus 500 mg/kg clarithromycin group, to 1.7 fold ($1,412 \times 10^3/\mu L$) in the 400 mg/kg AZT plus 500 mg/kg clarithromycin group, greater than the mean ($838 \times 10^3/\mu L$) for the controls. Biologically significant alterations in platelet counts did not occur in female-B mice.

Significant (P<0.05) alterations in leukocyte values (Table B1) did not occur in any of the male or female groups treated with combinations of AZT and clarithromycin.

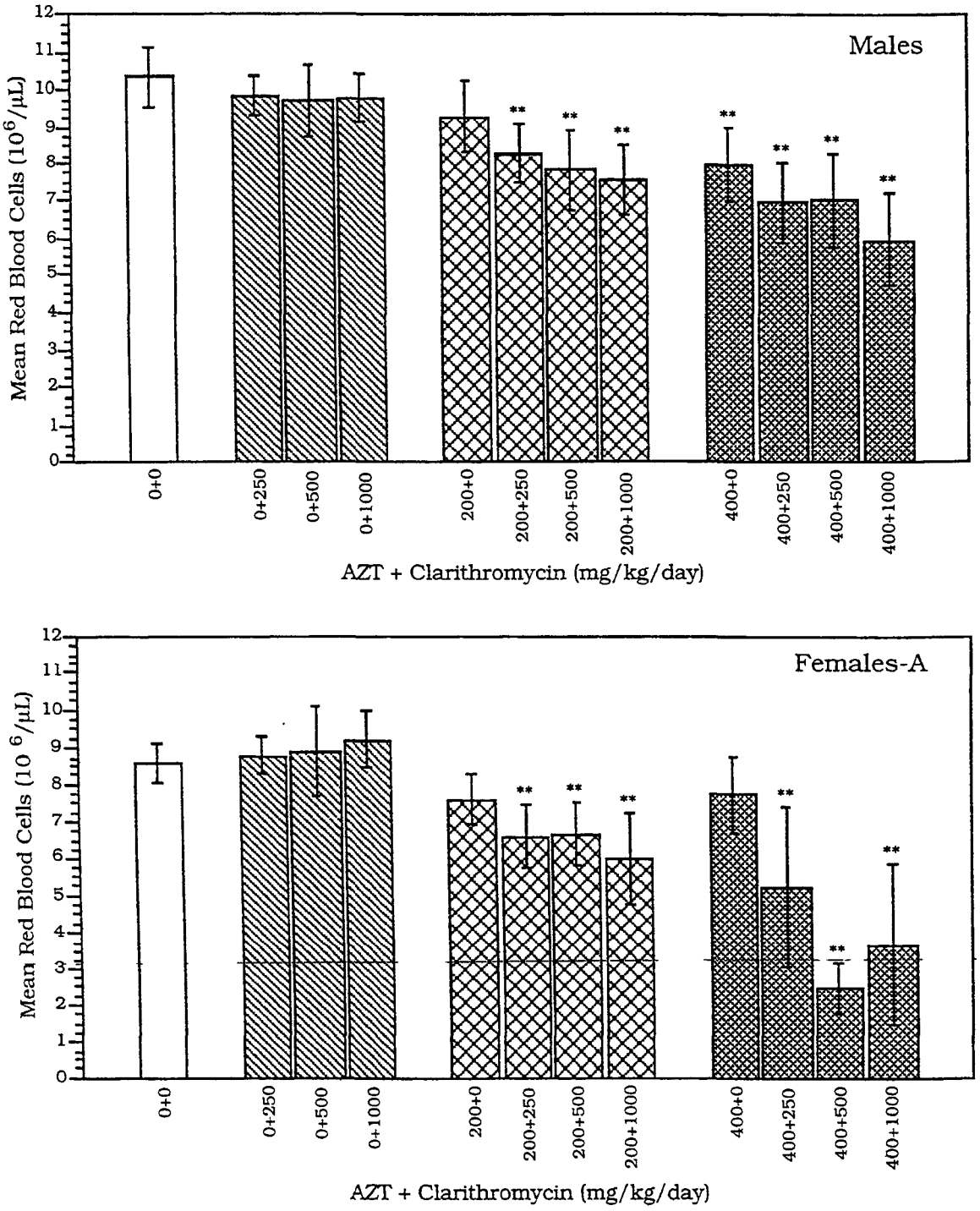


FIGURE 5
Mean Red Blood Cell Values for Male and Female-A Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations
[Bars indicate standard deviation, ** = significantly different ($P<0.01$) from control group using Dunnett's procedure.]

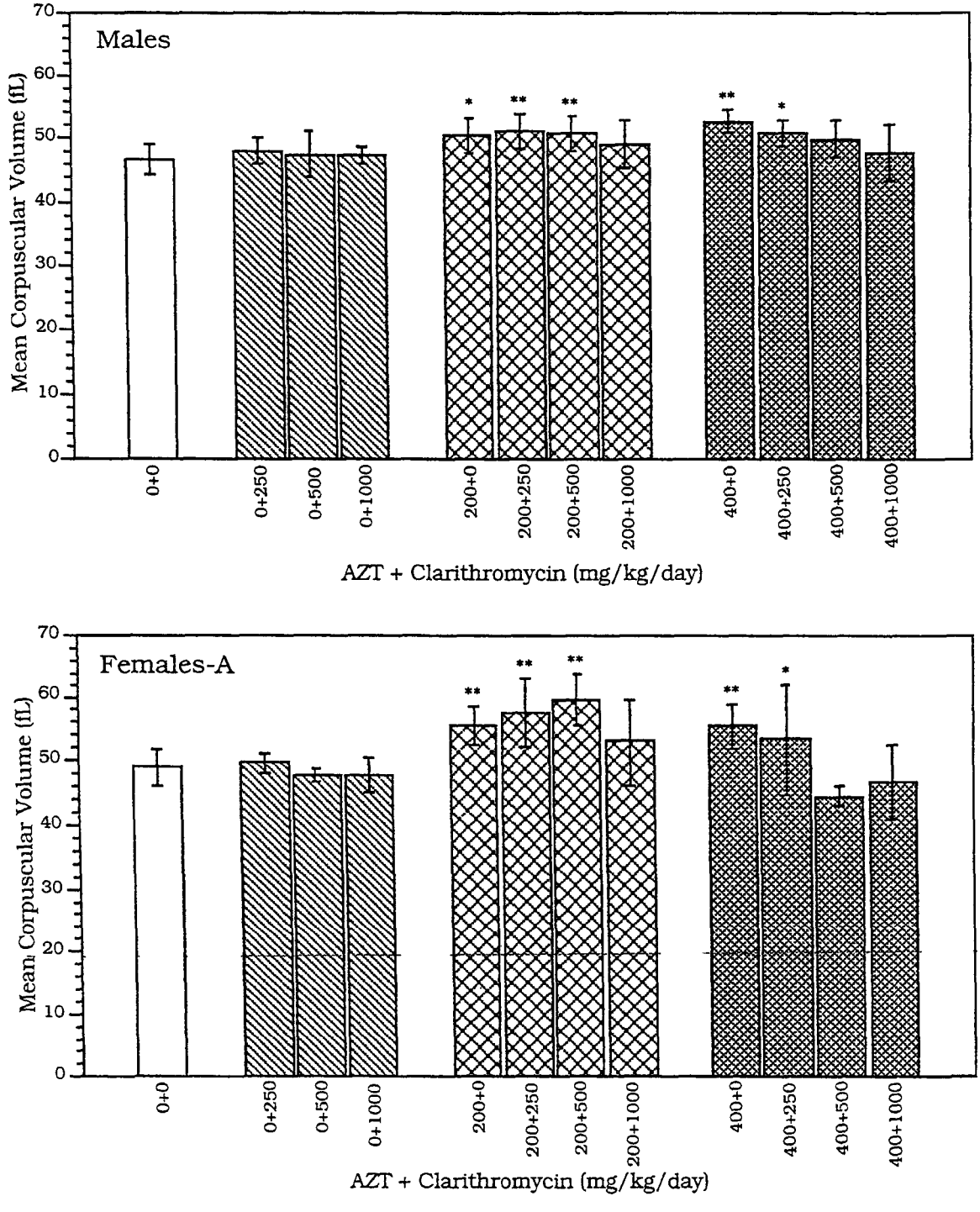


FIGURE 6
Mean Mean Corpuscular Volume for Male and Female-A Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations
[Bars indicate standard deviation, * = significantly different (P<0.05) from control group using Dunnett's procedure, ** = significantly different (P<0.01) from control group using Dunnett's procedure.]

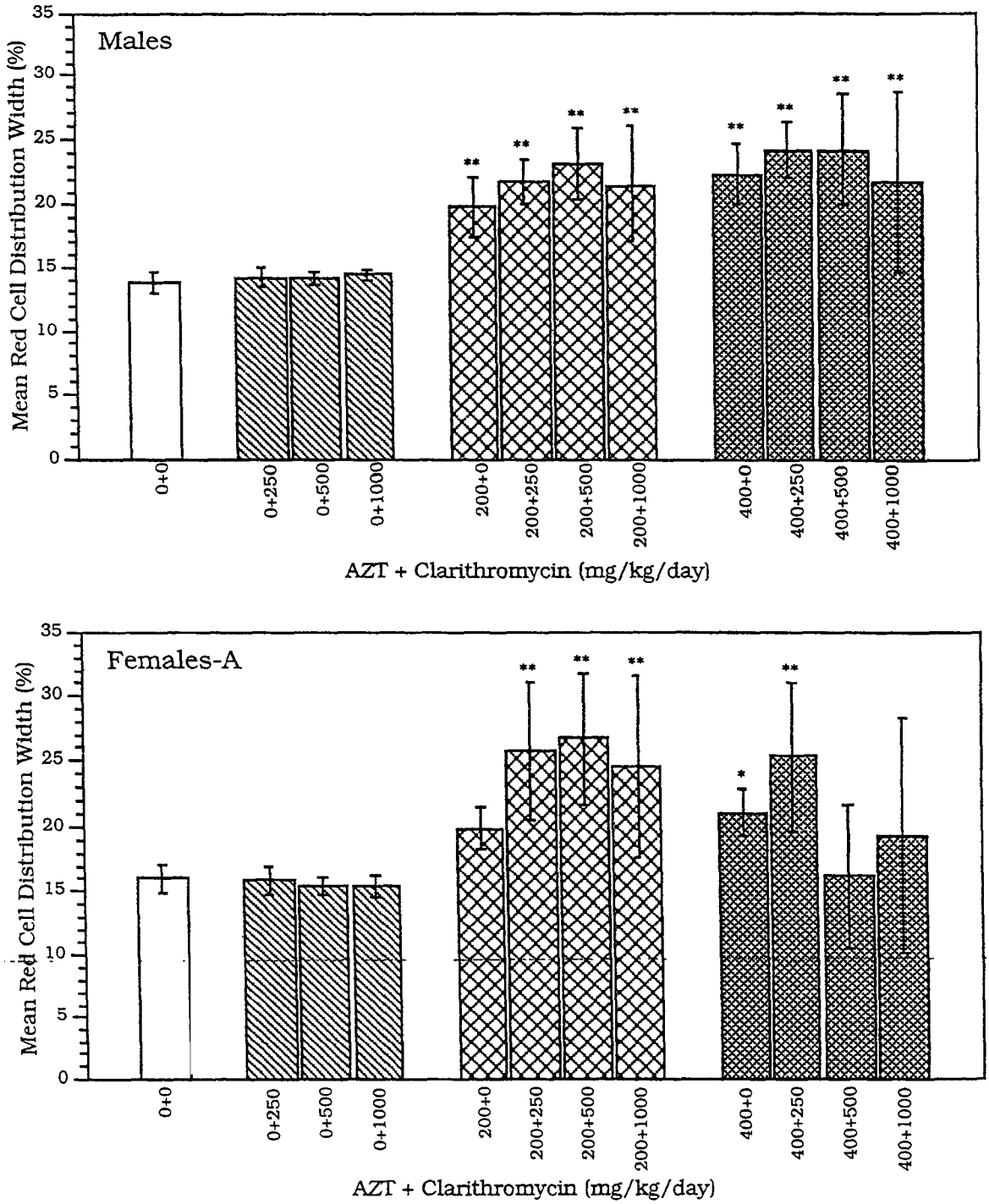


FIGURE 7
Mean Red Cell Distribution Width of Male and Female-A Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations
[Bars indicate standard deviation, * = significantly different ($P<0.05$) from control group using Dunnett's procedure, ** = significantly different ($P<0.01$) from control group using Dunnett's procedure.]

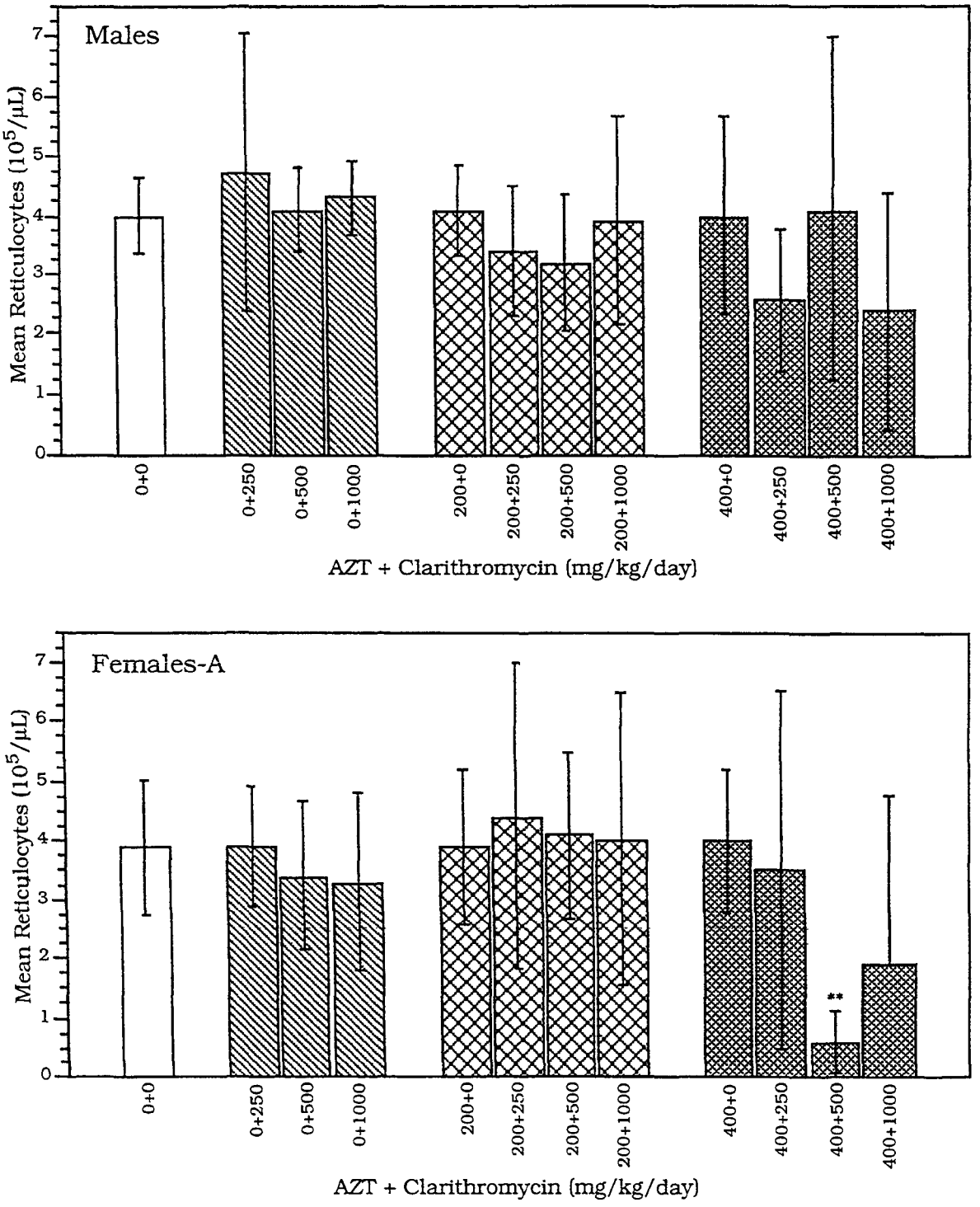


FIGURE 8
Mean Reticulocyte Values for Male and Female-A Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations
[Bars indicate standard deviation, ** = significantly different ($P<0.01$) from control group using Dunnett's procedure.]

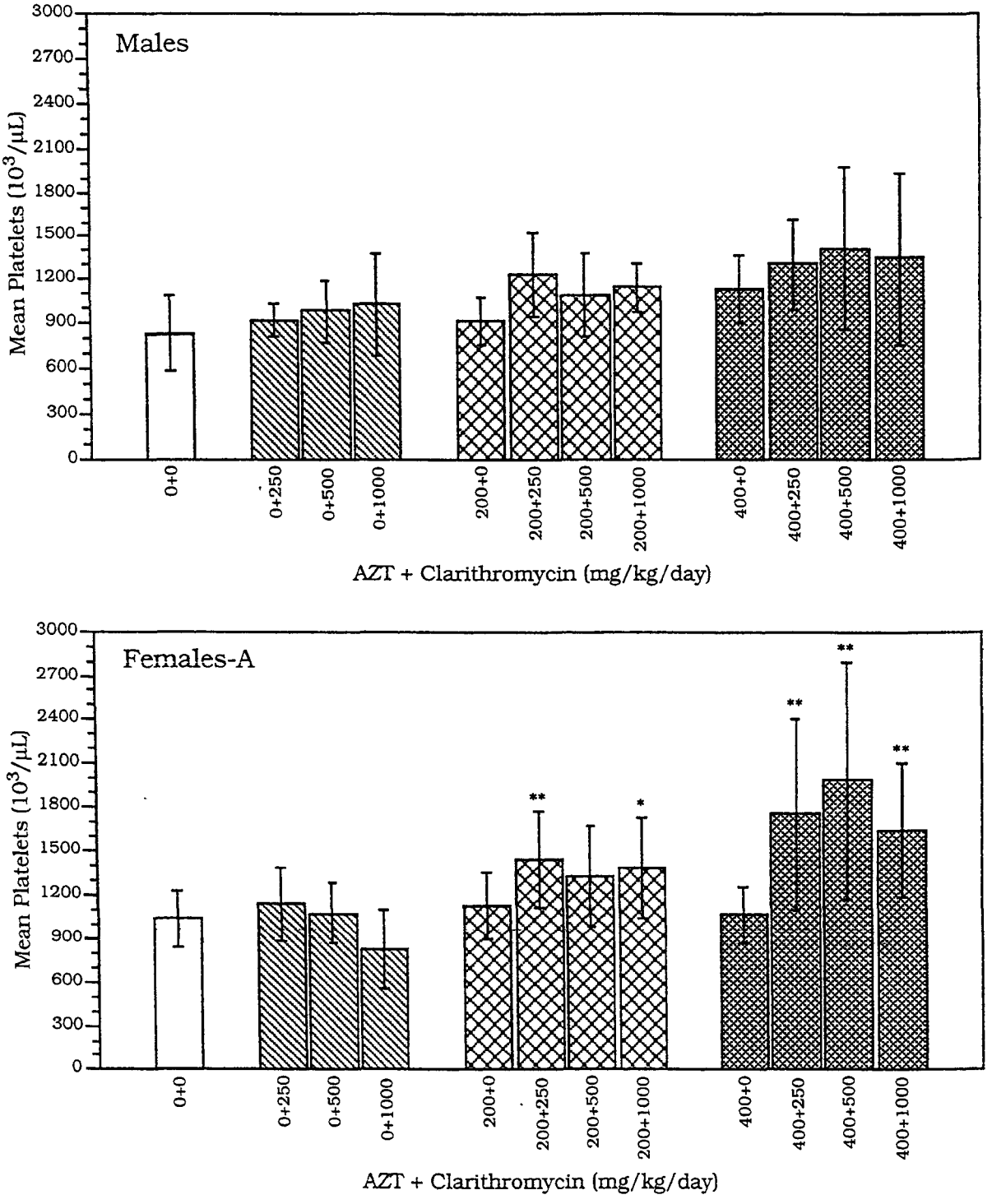


FIGURE 9

Mean Platelet Values for Male and Female-A Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations

[Bars indicate standard deviation, * = significantly different (P<0.05) from the control group using Dunnett's procedure, ** = significantly different (P<0.01) from control group using Dunnett's procedure.]

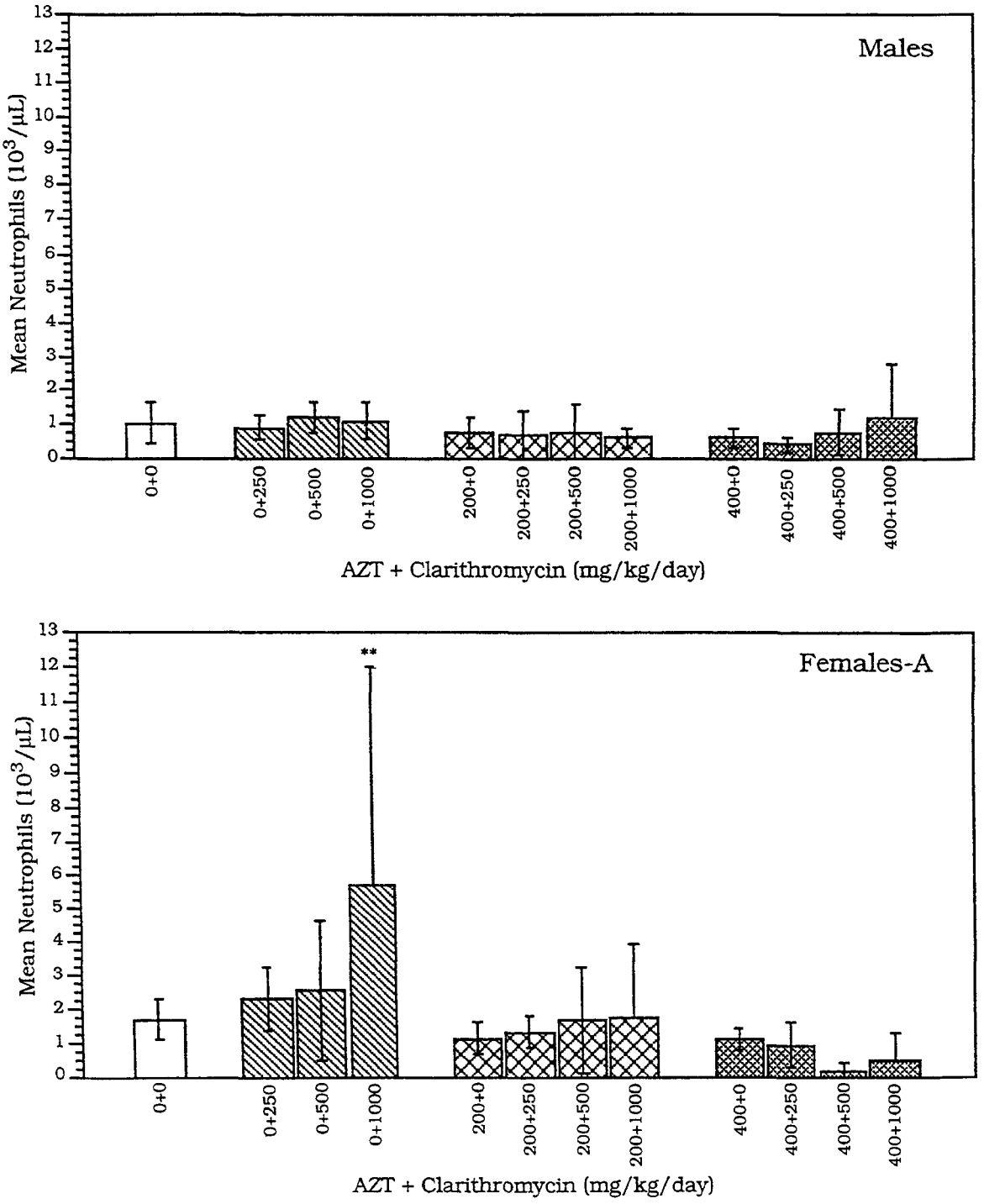


FIGURE 10
Mean Segmented Neutrophil Values for Male and Female-A Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations
[Bars indicate standard deviation, ** = significantly different (P<0.01) from control group using Dunnett's procedure.]

Clinical Chemistry

AZT Alone

Administration of 200 or 400 mg/kg AZT to male and female-A mice did not result in any treatment-related changes in serum chemistry parameters (Table B2).

Clarithromycin Alone

Administration of 250 or 500 mg/kg clarithromycin alone to male and female-A mice was not associated with any treatment-related alterations in any of the clinical chemistry parameters (Table B2). Significant alterations ($P<0.05$) in clinical chemistry parameters did not occur in the male group treated with 1,000 mg/kg clarithromycin for approximately 20 days. For the female-A group treated with 1,000 mg/kg clarithromycin for approximately 30 days, a slight increase in blood urea nitrogen (BUN) and alanine aminotransferase (ALT) values occurred. When compared to the female-A control group, with a BUN value of 18.3 mg/dL, the 1.3-fold increase (23.5 mg/dL, $P<0.01$) in the BUN value in the 1,000 mg/kg group was not believed to be biologically significant because a corresponding increase ($P<0.05$) in the creatinine value did not occur. Similarly, the 1.4-fold increase (52 U/L, $P<0.05$) in the ALT value in the 1,000 mg/kg female-A group, compared to 36 U/L in the control group, was not accompanied by increases in alkaline phosphatase, aspartate aminotransferase, or bile acid values indicative of liver toxicity.

AZT and Clarithromycin Combinations

Increases in clinical chemistry parameters (Table B2) indicative of kidney and liver toxicity did not occur in male or female-A mice treated with 200 or 400 mg/kg AZT in combination with 250, 500, or 1,000 mg/kg clarithromycin. Respective mean BUN values for female-A groups treated with 400 mg/kg AZT plus 250, 500, or 1,000 mg/kg clarithromycin were approximately 1.3 fold (24.2 mg/dL, $P<0.01$), 1.5 fold (27.8 mg/dL, $P<0.01$), and 1.3 fold (24.4 mg/dL, $P<0.05$) greater than the mean control value (18.3 mg/dL). These mild elevations in BUN values were not believed to be indicative of renal toxicity because corresponding elevations ($P<0.05$) in creatinine values did not occur. The statistically significant decrease ($P<0.05$) in ALT activity in the female-A group treated with 400 mg/kg AZT plus 500 mg/kg clarithromycin was not considered biologically significant.

Plasma Concentrations of AZT and Clarithromycin

AZT

A summary of plasma concentrations of AZT and its metabolites β-D-glucuronide of AZT (GAZT) and 3′-amino-3′-deoxythymidine (AMT) at 30, 60, 120, and 240 minutes after the last gavage dose is presented in Table 2 for male mice and in Table 3 for the pregnant female-A mice. The values for male mice are means of two samples

from two different mice and those for females are means of up to three samples from up to three different mice at each time point.

Male Mice

Administration of 400 mg/kg AZT alone resulted in plasma AZT concentrations of 141.5 to 0.96 μg/mL, GAZT concentrations of 4.16 μg/mL to nondetectable (ND, < 0.2 μg/mL) levels, and AMT concentrations of 1.32 μg/mL to nondetectable levels. Administration of 400 mg/kg AZT and 250 mg/kg clarithromycin resulted in plasma AZT concentrations of 48.0 to 2.3 μg/mL, GAZT concentrations of 1.10 μg/mL to nondetectable levels, and AMT concentrations of 0.60 to 0.23 μg/mL. Administration of 400 mg/kg AZT and 500 mg/kg clarithromycin resulted in plasma AZT concentrations of 31.0 to 2.6 μg/mL, GAZT concentrations of 0.61μg/mL to nondetectable levels, and AMT concentrations of 0.57 to 0.16 μg/ml. The highest dose combination, 400 mg/kg AZT and 1,000 mg/kg clarithromycin resulted in plasma AZT concentrations of 13.2 to 3.3 μg/mL, GAZT concentrations of 0.32 μg/mL to nondetectable levels, and AMT concentrations of 0.37 to 0.25 μg/mL from 30 to 240 minutes after the last dose in mice previously treated daily for approximately 20 days. The plasma 3′-amino-3′-deoxythymidine glucuronide (GAMT) concentrations in the male samples were at less than the detectable levels (<0.2μg/mL). Peak plasma concentrations of AZT in male mice decreased as the dose of clarithromycin increased, which is similar to the drug interaction previously described in acquired immunodeficiency syndrome patients treated simultaneously with AZT and clarithromycin (Polis *et al.*, 1997).

Female-A Mice

Administration of 400 mg/kg AZT resulted in plasma AZT concentrations of 101.5 to 3.2 μg/mL, GAZT concentrations of 1.05 μg/mL to nondetectable levels, and AMT concentrations of 1.65 to 0.40 μg/mL. Oral administration of 400 mg/kg AZT and 250 mg/kg clarithromycin resulted in plasma AZT concentrations of 70.0 to 3.8 μg/mL, GAZT concentrations of 0.62 μg/mL to nondetectable levels, and AMT concentrations of 1.31 to 0.48 μg/mL. Administration of 400 mg/kg AZT and 500 mg/kg clarithromycin resulted in plasma AZT concentrations of 56.1 to 6.5 μg/mL, GAZT concentrations of 0.51 μg/mL to nondetetable levels, and AMT concentrations of 0.72 to 0.24 μg/mL. The highest dose combination of 400 mg/kg AZT and 1,000 mg/kg clarithromycin resulted in plasma AZT concentrations of 41.7 to 4.1 μg/mL, GAZT concentrations of 0.41 μg/mL to nondetectable levels, and AMT concentrations of 0.81 to 0.41 μg/mL from 30 to 240 minutes after the last dose in mice previously treated daily for approximately 30 days. The plasma GAMT concentrations in the female-A samples were at less than the detectable levels. As in the male mice, peak plasma concentrations of AZT in female-A mice decreased as the oral dose of clarithromycin increased; however, plasma levels in general were higher in females than in males.

Clarithromycin

A summary of plasma concentrations of clarithromycin at 30, 60, 120, and 240 minutes after gavage administration of the last dose is presented in Table 2 for male mice and in Table 3 for pregnant female-A mice.

Male Mice

Clarithromycin alone at 250 mg/kg per day resulted in plasma clarithromycin concentrations of 2.74 to 1.30 μg/mL. Administration in of 400 mg/kg AZT and 250 mg/kg clarithromycin resulted in plasma clarithromycin concentrations of 3.43 to 1.92 μg/mL. Clarithromycin alone at 500 mg/kg resulted in plasma clarithromycin concentrations of 2.32 to 1.39 μg/mL, and 500 mg/kg combined with 400 mg/kg AZT resulted in plasma clarithromycin concentrations of 2.61 to 1.94 μg/mL. Clarithromycin at 1,000 mg/kg resulted in plasma concentrations of 4.34 to 1.6 μg/mL, and this amount of clarithromycin with 400 mg/kg AZT resulted in plasma clarithromycin concentrations of 4.77 to 1.81 μg/mL from 30 to 240 minutes after gavage administration of the last dose to mice previously treated daily for approximately 20 days.

Female-A Mice

Oral administration of clarithromycin alone at 250 mg/kg resulted in plasma clarithromycin concentrations of 15.6 to 10.31 μg/mL. Administration of 400 mg/kg AZT and 250 mg/kg clarithromycin resulted in plasma clarithromycin concentrations of 19.35 to 10.94 μg/mL. Clarithromycin at 500 mg/kg resulted in plasma clarithromycin concentrations of 13.72 to 9.35 μg/ml, and 500 mg/kg combined with 400 mg/kg AZT resulted in plasma clarithromycin concentrations of 19.65 to 16.2 μg/mL. Clarithromycin at 1,000 mg/kg resulted in plasma concentrations of 17.60 to 9.48 μg/mL, and 1,000 mg/kg with 400 mg/kg AZT resulted in plasma clarithromycin concentrations of 21.2 to 4.8 μg/mL from 30 to 240 minutes after gavage administration of the last dose to pregnant mice previously treated daily for approximately 30 days. In general, plasma concentrations of clarithromycin were higher in female-A mice than in male mice after oral dosing with clarithromycin alone or clarithromycin in combination with AZT.

TABLE 2
Concentrations of AZT, AZT Metabolites, and Clarithromycin in Plasma Samples of Male Swiss (CD-1®) Mice Treated with AZT and/or Clarithromycin for Approximately 20 Days[a]

Dose[b]	Sampling Time[c] (minutes)	AZT (μg/mL)	GAZT[d] (μg/mL)	AMT[e] (μg/mL)	Clarithromycin (μg/mL)
400 + 0	30	141 5	4 16	1 32	NA
	60	19 8	0 35	0 51	NA
	120	19 6	0 30	1 06	NA
	240	0 96	ND	0 16	NA
0 + 250	30	NA	NA	NA	1 30
	60	NA	NA	NA	1 65
	120	NA	NA	NA	2 74
	240	NA	NA	NA	2 63
0 + 500	30	NA	NA	NA	1 46
	60	NA	NA	NA	1 39
	120	NA	NA	NA	2 32
	240	NA	NA	NA	1 90
0 + 1,000	30	NA	NA	NA	2 96
	60	NA	NA	NA	1 60
	120	NA	NA	NA	2 34
	240	NA	NA	NA	4 34
400 + 250	30	48 0	1 10	0 60	1 96
	60	15 8	0 30	0 40	2 50
	120	10 7	0 30	0 23	3 43
	240	2 3	ND	0 60	1 92
400 + 500	30	31 0	0 61	0 57	2 61
	60	13 5	0 13	0 28	1 98
	120	6 3	0 11	0 16	1 94
	240	2 6	ND	0 34	2 48
400 + 1,000	30	13 2	0 19	0 25	1 81
	60	16 9	0 32	0 30	4 26
	120	3 3	ND	0 34	3 33
	240	5 1	ND	0 37	4 77

NA = not applicable, ND = not detected
[a] Mean of samples from two mice
[b] AZT + clarithromycin in mg/kg per day
[c] Minutes after the last dose
[d] β-D-glucuronide of AZT
[e] 3 -Amino-3 -deoxythymidine

TABLE 3
Concentrations of AZT, AZT Metabolites, and Clarithromycin in Plasma Samples of Female Swiss (CD-1®) Mice Treated with AZT and/or Clarithromycin for Approximately 30 Days Before and During Pregnancy[a]

Dose[b]	Sampling Time[c] (minutes)	AZT (μg/mL)	GAZT[d] (μg/mL)	AMT[e] (μg/mL)	Clarithromycin (μg/mL)
400 + 0	30	101 5	1 05	0 82	NA
	60	29 8	0 35	0 40	NA
	120	13 7	ND	0 76	NA
	240	3 2	ND	1 65	NA
0 + 250	30	NA	NA	NA	14 60
	60	NA	NA	NA	10 97
	120	NA	NA	NA	15 60
	240	NA	NA	NA	10 31
0 + 500	30	NA	NA	NA	13 02
	60	NA	NA	NA	11 90
	120	NA	NA	NA	9 35
	240	NA	NA	NA	13 72
0 + 1,000	30	NA	NA	NA	12 35
	60	NA	NA	NA	15 30
	120	NA	NA	NA	17 60
	240	NA	NA	NA	9 48
400 + 250	30	70 0	0 62	1 03	14 80
	60	36 2	0 31	0 50	12 30
	120	6 2	ND	0 48	19 35
	240	3 8	ND	1 31	10 94
400 + 500	30	56 1	0 51	0 61	16 70
	60	34 7	0 33	0 72	19 65
	120	7 7	ND	0 24	18 50
	240	6 5	ND	0 68	16 20
400 + 1,000	30	41 7	0 33	0 49	6 45
	60	34 6	0 41	0 41	4 80
	120	13 6	ND	0 44	21 20
	240	4 1	ND	0 81	19 70

NA = not applicable, ND = not detected
[a] Mean of samples from one to three mice
[b] AZT + clarithromycin in mg/kg per day
[c] Minutes after the last dose
[d] β-D-glucuronide of AZT
[e] 3 -Amino-3 -deoxythymidine

NECROPSY FINDINGS

Necropsy findings attributed to treatment with one or both drugs were seen in male mice, female-A mice, and female-B mice (Table C1). Necropsy findings in male mice that appeared to be treatment related consisted of pale carcass, mottled or pale kidneys, and small thymus. Possible treatment-related findings in female-A mice were small thymus, enlarged spleen, mottled or pale kidneys, pale carcass, dilatation of the stomach, and mottled stomach. The most significant finding in female-B mice was enlarged spleen. Deformed bone, including the ventral aspect of the thoracic vertebrae or ribs near their junction, occurred randomly in all groups and was attributed to mechanical irritation caused by the gavage needle during dosing.

HISTOPATHOLOGIC OBSERVATIONS

Major target organs included the liver, spleen, bone marrow, kidney, lymph nodes, thymus, brain, and heart. Selected photomicrographs of bone marrow from a control mouse and a treated mouse are shown in Plates 1 and 2.

Male and Female-A Mice

Liver Lesions

Male and female-A mice treated with AZT alone, clarithromycin alone, or combinations of AZT and clarithromycin developed cytoplasmic alteration in the liver (Table 4). This cellular change consisted of a more homogeneous, eosinophilic appearance to the hepatocyte cytoplasm accompanied by a concurrent loss of the fine irregularly shaped clear spaces normally attributed to the presence of glycogen in routine hematoxylin and eosin (H&E) preparations. There was also an absence of round, clear cytoplasmic vacuoles normally attributed to the presence of fat. This morphological alteration evident in the cytoplasm of hepatocytes likely represents a physiological response associated with weight loss, as opposed to a direct toxic cellular response to the test articles. The morphological change appeared to initially occur in the centrilobular areas adjacent to the central veins and subsequently involve the remaining zones of the liver lobule. Hepatocellular cytoplasmic alteration was graded for severity using the following criteria:

> *Minimal severity* - approximately 10% or less of hepatocytes, predominately around the central vein, with cytoplasmic alteration
>
> *Mild severity* - approximately 11% to 25% of hepatocytes with cytoplasmic alteration, possibly involving all zones of the lobule. This degree of severity was usually accompanied by other more significant changes such as cytoplasmic vacuolization or necrosis.

For male and female-A mice treated with AZT alone, the incidence of hepatocellular cytoplasmic alteration was low, and the degree of severity was considered minimal in all dose groups of both sexes. This liver lesion was not associated with any significant alterations in any of the clinical pathology parameters and was not statistically significant. For the male groups treated with AZT and clarithromycin combinations, the incidence was low, and significant increases ($P<0.05$) in severity grades did not occur (Table 5). Compared to the untreated control group, a significant ($P<0.01$) increase in severity occurred in the female-A group treated with 1,000 mg/kg clarithromycin alone. Compared to the groups treated with fixed levels of AZT alone (200 or 400 mg/kg), significant ($P<0.01$) increases in severity grades occurred in female-A groups receiving 200 mg/kg AZT plus 500 or 1,000 mg/kg clarithromycin and 400 mg/kg AZT plus 250, 500, or 1,000 mg/kg clarithromycin. The greater incidence of hepatocellular cytoplasmic alteration in female-A mice compared to the males corresponds with the increased duration of treatment.

A lesion in the liver of male and female-A mice associated with the highest dose of clarithromycin was cytoplasmic vacuolization (Table 4). The cytoplasmic vacuoles appeared to be largest and most prominent in the Kupffer cells, but also occurred to a lesser degree in the cytoplasm of hepatocytes. Cytoplasmic vacuolization of the liver was graded based on the following criteria:

> *Minimal severity* - utilizing a 4× objective and a 10× eyepiece, occasional vacuoles, approximately the same size as the hepatocyte nuclei, were found randomly distributed throughout the liver. Cytoplasmic vacuoles were present in approximately 10% or less of Kupffer cells
>
> *Mild severity* - utilizing a 4× objective and a 10× eyepiece, cytoplasmic vacuolization was evident in essentially all liver lobules. Irregular-shaped, clear spaces typical of glycogen in hepatocyte cytoplasm were replaced by round, clear cytoplasmic vacuoles. Cytoplasmic vacuoles were present in approximately 11% to 25% of Kupffer cells
>
> *Moderate severity* - utilizing a 4× objective and a 10× eyepiece, cytoplasmic vacuolization was uniformly present in all liver lobules. Irregular-shaped spaces typical of glycogen in the hepatocyte cytoplasm were replaced by round, clear cytoplasmic vacuoles. Cytoplasmic vacuoles were present in approximately 26% to 50% of the Kupffer cells.

The incidence of cytoplasmic vacuolization was similar in male and female-A mice. The lesion did not occur in groups treated with 250 or 500 mg/kg clarithromycin alone or in combination with AZT. Cytoplasmic vacuolization occurred with a low incidence in female-A mice treated with 200 mg/kg AZT plus 1,000 mg/kg clarithromycin and in male and female-A groups treated with 1,000 mg/kg clarithromycin alone and 400 mg/kg AZT plus 1,000 mg/kg clarithromycin. Combination therapy did not appear to enhance the incidence or severity of cytoplasmic vacuolization in the liver.

Treatment-related necrosis of hepatocytes (Table 4) also occurred in female-A mice treated with 1,000 mg/kg clarithromycin alone or in combination with AZT. The cellular necrosis was manifested by increased eosinophilia of disrupted cytoplasm and karyorrhexis and pyknosis of nuclear material. The necrosis occurred in a zonal pattern predominately around the central veins. Criteria for the grading of hepatocyte necrosis were as follows:

Minimal severity - occasional (<10%) necrotic cells present in a centrilobular pattern

Mild severity - approximately 11% to 25% of hepatocytes in centrilobular areas undergoing necrosis.

Hepatocyte necrosis of minimal severity occurred in two female-A mice treated with 1,000 mg/kg clarithromycin alone and in three female-A mice each in the groups treated with 200 or 400 mg/kg AZT plus 1,000 mg/kg clarithromycin. Necrosis was not diagnosed in any of the male mice treated with combinations of AZT and clarithromycin or in the female-A groups treated with AZT in combination with 250 or 500 mg/kg clarithromycin. The minimal necrosis of hepatocytes may reflect an anoxic change secondary to anemia.

TABLE 4
Histopathological Alterations in the Liver in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations in Swiss (CD-1®) Mice

	Cytoplasmic Alteration[b]		Necrosis[b]		Cytoplasmic Vacuolization[b]	
Dose[a]	Males	Females-A	Males	Females-A	Males	Females-A
0 + 0	0/10	0/20	0/10	0/20	0/0	0/20
0 + 250	0/10	3/20 (1 0)	0/10	0/20	0/10	0/20
0 + 500	3/10 (1 0)	2/20 (1 0)	0/10	0/20	0/10	0/20
0 + 1,000	2/10 (1 0)	10/20 (1 3)	0/10	2/20 (1 0)	1/10 (1 0)	2/20 (2 5)
200 + 0	3/10 (1 0)	1/20 (1 0)	0/10	0/20	0/10	0/20
200 + 250	6/10 (1 0)	8/20 (1 0)	0/10	0/20	0/10	0/20
200 + 500	3/10 (1 0)	16/20 (1 0)	0/10	0/20	0/10	0/20
200 + 1,000	2/10 (1 0)	18/20 (1 1)	0/10	3/20 (1 0)	0/10	3/20 (1 0)
400 + 0	2/10 (1 0)	2/20 (1 0)	0/10	0/20	0/10	0/20
400 + 250	1/10 (2 0)	13/20 (1 0)	0/10	0/20	0/10	0/20
400 + 500	4/10 (1 3)	20/20 (1 0)	0/10	0/20	0/10	0/20
400 + 1,000	4/10 (1 0)	19/20 (1 1)	0/10	3/20 (1 0)	1/10 (1 0)	2/20 (1 0)

[a] AZT + clarithromycin in mg/kg per day

[b] Number of animals in group with lesion/number of animals examined (mean severity for mice with lesion, grade 1=minimal, 2=mild, 3=moderate, 4=marked)

TABLE 5
Statistical Analysis of Mean Severity of Hepatocellular Cytoplasmic Alteration in Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Clarithromycin Combinations

	Severity in Males[a]			Severity in Females-A[a]		
Dose[b]	Mean[c]	S.E.	Ratio[e]	Mean[d]	S.E.	Ratio[e]
0 + 0	0 000	0 000	NA	0 000	0 000	NA
0 + 250	0 000	0 000	—	0 150	0 082	—
0 + 500	0 300	0 153	—	0 100	0 069	—
0 + 1,000	0 200	0 133	—	**0 650	0 182	—
	Trend[f]	+ (P=0 057)		Trend[f]	+ (P=0 001)	
	Test Used[g]	Dunn		Test Used[g]	Shirley	
200 + 0	0 300	0 153	NA	0 050	0 050	NA
200 + 250	0 600	0 163	200	0 400	0 112	800
200 + 500	0 300	0 153	100	**0 800	0 092	1,600
200 + 1,000	0 200	0 133	67	**1 000	0 103	2,000
	Trend[f]	- (P=0 380)		Trend[f]	+ (P<0 001)	
	Test Used[g]	Dunn		Test Used[g]	Shirley	
400 + 0	0 200	0 133	NA	0 100	0 069	NA
400 + 250	0 200	0 200	100	**0 650	0 109	650
400 + 500	0 500	0 224	250	**1 000	0 000	1,000
400 + 1,000	0 400	0 163	200	**1 000	0 073	1,000
	Trend[f]	+ (P=0 198)		Trend[f]	+ (P<0 001)	
	Test Used[g]	Dunn		Test Used[g]	Shirley	

[a] Severity grade presented as mean severity (1=minimal, 2=mild, 3=moderate, 4=marked) and standard error (S E)
[b] AZT + clarithromycin in mg/kg per day
[c] n=10
[d] n=20
[e] (Dosed group mean /control group mean) × 100, NA = not applicable, — = ratio cannot be calculated
[f] Direction and significance of trend (Jonckheere's test)
[g] Multiple comparisons test comparing dose group to control group
* Significantly different from the control group (P<0 05)
** Significantly different from the control group (P<0 01)

Spleen Lesions

A treatment-related alteration in the spleen of male and female-A mice (Table 6) treated with 200 or 400 mg/kg AZT consisted of hematopoietic cell proliferation evident in the red pulp. The hematopoiesis involved primarily cells of the erythroid and megakaryocytic series and was believed to have been a compensatory response to the slight anemia induced by AZT. Extramedullary hematopoiesis (grade 0 for hematopoietic cell proliferation) consisting of megakaryocytes, foci of erythroid precursors, and large granulocytic precursors is a normal

occurrence in the red pulp of the spleen of mice (Maronpot, 1999). Hematopoietic cell proliferation of the spleen was graded for severity based on the following criteria:

> *Minimal severity* - utilizing a 4× objective and a 10× eye piece, the width of the spleen usually approached or slightly exceeded the field of view. Approximately 10% or less of the red pulp was occupied by erythropoietic cells and/or megakaryocytes
>
> *Mild severity* - utilizing a 4× objective and a 10× eye piece, the width of the spleen usually approached or slightly exceeded the field of view. Approximately 11% to 25% of the red pulp was occupied by erythropoietic cells and/or megakaryocytes
>
> *Moderate severity* - the width of the spleen exceeded the field of view when observed with a 4× objective and a 10× eyepiece. Approximately 26% to 50% of the red pulp was occupied by erythropoietic cells and/or megakaryocytes.

The incidence of hematopoietic cell proliferation in male mice increased as dose levels of AZT increased; however, the degree of severity was considered minimal for both treatment groups. For the female-A mice, the incidence and the severity increased slightly as AZT dose levels increased. For the male groups treated with 250 or 1,000 mg/kg clarithromycin alone, hematopoietic cell proliferation occurred with a low incidence; however, when compared to the untreated control group, a significant increase ($P<0.05$) in mean severity occurred in the male group treated with 1,000 mg/kg clarithromycin alone (Table 7). For the female-A groups treated with 250, 500, or 1,000 mg/kg clarithromycin, the incidence and severity of hematopoietic cell proliferation were slightly greater than for the control group. Statistically significant ($P<0.05$) increases in severity grades, however, did not occur. For the male groups treated with 200 mg/kg AZT plus clarithromycin, the incidence of hematopoietic cell proliferation was similar to the group treated with 200 mg/kg AZT alone. For male groups treated with 400 mg/kg AZT in combination with clarithromycin, the incidence of hematopoietic cell proliferation was lower than for the group treated with 400 mg/kg AZT alone. Statistically significant ($P<0.05$) alterations in severity grades, however, did not occur. For the female-A groups treated with 200 mg/kg AZT plus clarithromycin, the incidence of hematopoietic cell proliferation was greater than for the female-A group treated with 200 mg/kg AZT alone. Compared to the female-A group treated with 200 mg/kg AZT alone, significant increases ($P<0.01$) in severity grades occurred in groups treated with 200 mg/kg AZT plus 500 or 1,000 mg/kg clarithromycin. For the female-A groups treated with 400 mg/kg AZT in combination with clarithromycin, the incidence of hematopoietic cell proliferation decreased as dose levels of clarithromycin increased. Compared to the group treated with 400 mg/kg AZT alone, significant declines ($P<0.01$) in mean severity grades occurred in groups treated with 400 mg/kg AZT plus 500 or 1,000 mg/kg clarithromycin. The lower hematopoietic cell proliferation in the higher-dosed combination groups was believed to reflect an inability of the spleen to adequately respond to the anemia that occurred in these groups.

A slight increase in the amount of hemosiderin deposition in the red pulp of the spleen (Table 6) occurred in male and female-A mice treated with AZT alone. Hemosiderin deposition was believed to be associated with the hematological alterations evident in the peripheral blood and likely resulted from an increased turnover rate of RBCs. Hemosiderin deposition in the spleen was graded for severity based on the following criteria:

> *Minimal severity* - utilizing a 4× objective in conjunction with a 10× eyepiece, a faint brown tint was suspected in the red pulp of the spleen. Higher magnification revealed pigment deposition in up to approximately 10% of the macrophages in the red pulp
>
> *Mild severity* - utilizing a 4× objective in conjunction with a 10× eyepiece, a faint brown tint was present in the red pulp of the spleen. Higher magnification revealed pigment deposition in approximately 11% to 25% of the macrophages in the red pulp
>
> *Moderat*e severity - a distinct brown to tan coloration was evident in the red pulp when viewed with a 4× objective and a 10× eyepiece. Higher magnification revealed pigment in approximately 26% to 50% or more of the macrophages in the splenic red pulp.

The presence of iron in the deposits of pigment was verified in representative animals from affected treatment groups by a positive staining reaction using a Prussian Blue stain (Sheehan and Rapchak, 1980). Increased hemosiderin pigmentation did not occur in male or female-A mice treated with clarithromycin alone. Combination therapy resulted in increased hemosiderin deposition in both sexes.

With dosages of 200 or 400 mg/kg AZT alone, the incidence of hemosiderin pigmentation in the spleen increased slightly in male and female-A mice as dose levels increased (Table 6). The degree of severity was graded as minimal to mild for all treatment groups of both sexes. Increased hemosiderin deposition did not occur in male or female-A groups treated with 250, 500, or 1,000 mg/kg clarithromycin alone (Table 6). For male and female-A mice treated with combination therapy, hemosiderin deposition in the spleen tended to increase as dose levels of AZT and clarithromycin increased and corresponded with the anemia indicated by the hematology parameters. Compared to the male group treated with 200 mg/kg AZT alone, a significant increase ($P<0.01$) in the severity occurred in the group treated with 200 mg/kg AZT plus 1,000 mg/kg clarithromycin (Table 7). When compared to the groups treated with 200 or 400 mg/kg AZT alone, significant increases ($P<0.01$) in severity occurred in all female-A groups receiving combination therapy (Table 8).

Depletion of splenic lymphoid follicles and red pulp occurred in the female-A groups treated with 1,000 mg/kg clarithromycin and in the male and female-A groups treated with AZT plus higher dosages of clarithromycin (Table 6). Depletion of the red pulp involved primarily cells of the erythrocytic series and was often accompanied by reticuloendothelial cells with large clear vacuoles in their cytoplasm. The vacuoles were often so extensive that the spleen appeared larger than normal. At times, the swollen reticuloendothelial cells appeared to be associated with sequestration of polymorphonuclear leukocytes in the sinusoids of the spleen. In general, depletion of the

red pulp usually occurred concurrently with cellular depletion of the white pulp or lymphoid follicles. Depletion of the red pulp and lymphoid follicles of the spleen was graded based on the following criteria:

> *Minimal severity* - depletion of approximately 10% or less of the normal cell population
>
> *Mild severity* - depletion of approximately 11% to 25% of the normal cell population with distinct vacuoles being evident in the red pulp when viewed with a 4× objective and a 10× eyepiece
>
> *Moderate severity* - depletion of approximately 26% to 50% of the normal cell population with distinct vacuoles being prominent in the red pulp when viewed with a 4× objective and a 10× eyepiece
>
> *Marked severity* - depletion of approximately 51% to 100% of the normal cell population and prominent vacuolated reticuloendothelial cells replacing the majority of the red pulp when viewed with a 4× objective and a 10× eyepiece.

Atrophy of the splenic red pulp and lymphoid follicles (Table 6) occurred with a low incidence in the female-A group treated with 1,000 mg/kg clarithromycin. Red pulp atrophy was diagnosed in only one male mouse in the group treated with 400 mg/kg AZT plus 1,000 mg/kg clarithromycin. For the female-A mice, however, the combination of 400 mg/kg AZT plus 500 mg/kg clarithromycin resulted in red pulp atrophy that was not found in the female-A group treated with 500 mg/kg clarithromycin alone, and the incidence and severity increased in the female-A group treated with 400 mg/kg AZT plus 1,000 mg/kg clarithromycin. Lymphoid follicle atrophy occurred with a low incidence in male mice treated with 400 mg/kg AZT plus 1,000 mg/kg clarithromycin and female-A groups treated with 200 or 400 mg/kg AZT plus 1,000 mg/kg clarithromycin. Lymphoid follicle atrophy tended to occur in the same groups that had red pulp atrophy.

TABLE 6
Histopathological Alterations in the Spleen in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations in Swiss (CD-1®) Mice

Dose[a]	Hematopoietic Cell Proliferation[b]		Hemosiderin Pigmentation[b]		Lymphoid Follicle Depletion[b]		Red Pulp Atrophy[b]	
	Males	Females-A	Males	Females-A	Males	Females-A	Males	Females-A
0 + 0	0/10	8/20 (1 0)	0/10	0/20	0/10	0/20	0/10	0/20
0 + 250	1/10 (2 0)	13/20 (1 2)	0/10	0/20	0/10	0/20	0/10	0/20
0 + 500	0/10	13/20 (1 1)	0/10	0/20	0/10	0/20	0/10	0/20
0 + 1,000	4/10 (1 0)	14/20 (1 1)	0/10	0/20	0/10	2/20 (2 5)	0/10	3/20 (2 0)
200 + 0	4/10 (1 0)	14/20 (1 1)	2/10 (1 0)	8/20 (1 0)	0/10	0/20	0/10	0/20
200 + 250	5/10 (1 0)	17/20 (1 1)	3/10 (1 0)	17/20 (1 0)	0/10	0/20	0/10	0/20
200 + 500	6/10 (1 5)	19/20 (1 5)	6/10 (1 0)	19/20 (1 1)	0/10	0/20	0/10	0/20
200 + 1,000	5/10 (1 2)	17/20 (2 0)	9/10 (1 8)	19/20 (1 5)	0/10	2/20 (2 0)	0/10	4/20 (1 8)
400 + 0	9/10 (1 0)	18/20 (1 3)	4/10 (1 0)	15/20 (1 0)	0/10	0/20	0/10	0/20
400 + 250	4/10 (1 3)	19/20 (1 5)	7/10 (1 0)	20/20 (1 8)	0/10	0/20	0/10	0/20
400 + 500	4/10 (1 8)	10/20 (1 1)	8/10 (1 3)	20/20 (2 2)	0/10	0/20	0/10	10/20 (1 2)
400 + 1,000	5/10 (1 6)	5/20 (2 4)	5/10 (1 4)	19/20 (1 9)	2/10 (2 0)	1/20 (3 0)	1/10 (4 0)	15/20 (1 7)

[a] AZT + clarithromycin in mg/kg per day

[b] Number of animals with lesion/number of animals examined (mean severity for mice with lesion, grade 1=minimal, 2=mild, 3=moderate, 4=marked)

TABLE 7
Statistical Analysis of Mean Severity of Hematopoietic Cell Proliferation in the Spleen of Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations

	Severity in Males[a]			Severity in Females-A[a]		
Dose[b]	Mean[c]	S.E.	Ratio[d]	Mean[e]	S.E.	Ratio[d]
0 + 0	0 000	0 000	NA	0 333	0 167	NA
0 + 250	0 200	0 200	—	0 750	0 143	225
0 + 500	0 000	0 000	—	0 700	0 128	210
0 + 1,000	*0 400	0 163	—	0 800	0 138	240
	Trend[f]	+ (P=0 026)		Trend[f]	+ (P=0 156)	
	Test Used[g]	Dunn		Test Used[g]	Dunn	
200 + 0	0 400	0 163	NA	0 750	0 123	NA
200 + 250	0 500	0 167	125	0 950	0 114	127
200 + 500	0 900	0 277	225	**1 400	0 169	187
200 + 1,000	0 600	0 221	150	**1 700	0 231	227
	Trend[f]	+ (P=0 368)		Trend[f]	+ (P<0 001)	
	Test Used[g]	Dunn		Test Used[g]	Shirley	
400 + 0	0 900	0 100	NA	1 200	0 138	NA
400 + 250	0 500	0 224	56	1 450	0 170	121
400 + 500	0 700	0 300	78	**0 550	0 135	46
400 + 1,000	0 800	0 291	89	**0 600	0 245	50
	Trend[f]	- (P=0 555)		Trend[f]	- (P<0 001)	
	Test Used[g]	Dunn		Test Used[g]	Shirley	

a Severity grade presented as mean severity (1=minimal, 2=mild, 3=moderate, 4=marked) and standard error (S E)
b AZT + clarithromycin in mg/kg per day
c n=10
d (Dosed group mean/control group mean) × 100, NA = not applicable, — = ratio cannot be calculated
e n=20
f Direction and significance of trend (Jonckheere's test)
g Multiple comparisons test comparing dose group to control group
* Significantly different from the control group (P<0 05)
** Significantly different from the control group (P<0 01)

TABLE 8
Statistical Analysis of Mean Severity of Hemosiderin Pigmentation in the Spleen of Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations

	Severity in Males[a]			Severity in Females-A[a]		
Dose[b]	Mean[c]	S.E.	Ratio[e]	Mean[d]	S.E.	Ratio[e]
0 + 0	0 000	0 000	NA	0 000	0 000	NA
0 + 250	0 000	0 000	—	0 000	0 000	—
0 + 500	0 000	0 000	—	0 000	0 000	—
0 + 1,000	0 000	0 000	—	0 000	0 000	—
	Trend[f]	(P=1 000)		Trend[f]	(P=1 000)	
	Test Used[g]	Dunn		Test Used[g]	Dunn	
200 + 0	0 200	0 133	NA	0 400	0 112	NA
200 + 250	0 300	0 153	150	**0 850	0 082	213
200 + 500	0 600	0 163	300	**1 050	0 088	263
200 + 1,000	**0 900	0 100	450	**1 400	0 134	350
	Trend[f]	+ (P=0 001)		Trend[f]	+ (P<0 001)	
	Test Used[g]	Shirley		Test Used[g]	Shirley	
400 + 0	0 400	0 163	NA	0 750	0 099	NA
400 + 250	0 700	0 153	175	**1 750	0 123	233
400 + 500	1 000	0 211	250	**2 200	0 092	293
400 + 1,000	0 700	0 260	175	**1 850	0 131	247
	Trend[f]	+ (P=0 227)		Trend[f]	+ (P<0 001)	
	Test Used[g]	Dunn		Test Used[g]	Shirley	

a Severity grade presented as mean severity (1=minimal, 2=mild, 3=moderate, 4=marked) and standard error (S E)
b AZT + clarithromycin in mg/kg per day
c n=10
d n=20
e (Dosed group mean/control group mean) × 100, NA = not applicable, — = ratio cannot be calculated
f Direction and significance of trend (Jonckheere's test)
g Multiple comparisons test comparing dose group to control group
* Significantly different from the control group (P<0 05)
** Significantly different from the control group (P<0 01)

Thymus Lesions

Atrophy of the thymus (Table 9) occurred primarily in male and female-A mice treated with clarithromycin alone or in combination with AZT and was considered to be compound related. This alteration was also believed to be related to stress. Thymic atrophy was graded for severity based on the following criteria:

Minimal severity - depletion of approximately 5% or less of cortical lymphocytes

Mild severity - depletion of approximately 6% to 20% of cortical lymphocytes

Moderate severity - depletion of approximately 21% to 50% of cortical lymphocytes.

For the male groups, the incidences of atrophy of the thymus (Table 9) were low and none of the severity grades (Table 10) were statistically significant ($P<0.05$). For the female-A group treated with 1,000 mg/kg clarithromycin, a significant ($P<0.05$) increase in severity occurred when compared to the untreated control group. Compared to the female-A group treated with 200 mg/kg AZT alone, a significant increase ($P<0.01$) in severity occurred in the group treated with 200 mg/kg AZT plus 1,000 mg/kg clarithromycin. When compared to the group treated with 400 mg/kg AZT alone, significant dose-related increases ($P<0.05$ to $P<0.01$) in severity occurred in female-A groups treated with 400 mg/kg AZT plus 250, 500, or 1,000 mg/kg clarithromycin.

TABLE 9
Histopathological Alterations in the Thymus in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations in Swiss (CD-1®) Mice

	Atrophy[b]			
Dose[a]	Males		Females-A	
0 + 0	0/10		0/20	
0 + 250	0/10		1/20	(1 0)
0 + 500	0/10		0/20	
0 + 1,000	1/10	(1 1)	6/20	(1 7)
200 + 0	0/10		1/20	(1 0)
200 + 250	0/9		1/18	(1.0)
200 + 500	0/10		0/20	
200 + 1,000	0/9		9/20	(1 6)
400 + 0	0/10		0/20	
400 + 250	1/10	(1 0)	4/20	(1 5)
400 + 500	3/10	(1 0)	5/19	(2.2)
400 + 1,000	3/10	(2 3)	11/18	(2 1)

[a] AZT + clarithromycin in mg/kg per day

[b] Number of animals with lesion/number of animals examined (mean severity for mice with lesion, grade 1=minimal, 2=mild, 3=moderate, 4=marked)

TABLE 10
Statistical Analysis of Mean Severity of Thymic Atrophy in Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations

	Severity in Males[a]				Severity in Females-A[a]			
Dose[b]	n	Mean	S.E.	Ratio[c]	n	Mean	S.E.	Ratio[c]
0 + 0	10	0 000	0 000	NA	20	0 000	0 000	NA
0 + 250	10	0 000	0 000	—	20	0 050	0 050	—
0 + 500	10	0 000	0 000	—	20	0 000	0 000	—
0 + 1,000	10	0 100	0 100	—	20	*0 500	0 212	—
		Trend[d]	+ (P=0 180)			Trend[d]	+ (P=0 006)	
		Test Used[e]	Dunn			Test Used[e]	Shirley	
200 + 0	10	0 000	0 000	NA	20	0 050	0 050	NA
200 + 250	9	0 000	0 000	—	18	0 056	0 056	111
200 + 500	10	0 000	0 000	—	20	0 000	0 000	0
200 + 1,000	9	0 000	0 000	—	20	**0 700	0 206	1,400
		Trend[d]	(P=1 000)			Trend[d]	+ (P=0 001)	
		Test Used[e]	Dunn			Test Used[e]	Shirley	
400 + 0	10	0 000	0 000	NA	20	0 000	0 000	NA
400 + 250	10	0 100	0 100	—	20	*0 300	0 164	—
400 + 500	10	0 300	0 153	—	19	*0 579	0 257	—
400 + 1,000	10	0 700	0 396	—	18	**1 278	0 289	—
		Trend[d]	+ (P=0 031)			Trend[d]	+ (P<0 001)	
		Test Used[e]	Dunn			Test Used[e]	Shirley	

[a] Severity grade presented as mean severity (1=minimal, 2=mild, 3=moderate, 4=marked) and standard error (S E)
[b] AZT + clarithromycin in mg/kg per day
[c] (Dosed group mean/control group mean) × 100, NA = not applicable, — = ratio cannot be calculated
[d] Direction and significance of trend (Jonckheere's test)
[e] Multiple comparisons test comparing dose group to control group
* Significantly different from the control group (P<0 05)
** Significantly different from the control group (P<0 01)

Bone Marrow Lesions

Depletion of the bone marrow (Table 11 and Plate 2) occurred primarily in male and female-A groups treated with combinations of AZT and clarithromycin. The bone marrow depletion occurred predominately in the erythrocytic series. Bone marrow depletion was graded for severity based upon the following criteria:

Minimal severity - depletion of approximately 5% or less of cells of the erythrocytic series

Mild severity - depletion of approximately 6% to 20% of cells of the erythrocytic series

Moderate severity - depletion of approximately 21% to 50% of cells of the erythrocytic series

Marked severity - depletion of greater than 50% of cells of the erythrocytic series.

Female-A mice treated for approximately 30 days appeared to be affected more severely than male mice, and the development of this lesion was believed to correspond with the increasing severity of anemia associated with the inability of the bone marrow to respond adequately. For the male mice treated for approximately 20 days, bone marrow depletion occurred only in groups treated with 200 or 400 mg/kg AZT plus 1,000 mg/kg clarithromycin. One female-A mouse treated with 1,000 mg/kg clarithromycin alone developed bone marrow depletion. For the female-A groups treated with 200 mg/kg AZT plus 1,000 mg/kg clarithromycin or 400 mg/kg AZT plus 250, 500, or 1,000 mg/kg clarithromycin, the incidence and severity of bone marrow depletion, in general, increased as dosage levels increased.

TABLE 11
Histopathological Alterations in the Bone Marrow in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations in Swiss (CD-1®) Mice

	Cellular Depletion[b]	
Dose[a]	Males	Females-A
0 + 0	0/10	0/20
0 + 250	0/10	0/20
0 + 500	0/10	0/20
0 + 1,000	0/10	1/20 (2 0)
200 + 0	0/10	0/20
200 + 250	0/10	0/20
200 + 500	0/10	0/20
200 + 1,000	3/10 (1 0)	6/20 (1 3)
400 + 0	0/10	0/20
400 + 250	0/10	7/20 (1 7)
400 + 500	0/10	18/20 (2 2)
400 + 1,000	4/10 (2 0)	15/20 (2 9)

[a] AZT + clarithromycin in mg/kg per day
[b] Number of animals with lesion/number of animals examined (mean severity for mice with lesion, grade 1=minimal, 2=mild, 3=moderate, 4=marked)

Kidney Lesions

Nephropathy (Table 12) was diagnosed in male and female-A mice treated with clarithromycin alone. Nephropathy included a variety of changes that occurred predominantly in the renal tubules, including necrosis, regeneration, and cytoplasmic vacuolization of tubular epithelial cells in the cortex. The necrosis was evident from the outer medullary zone and corticomedullary junction to the periphery of the cortex. Necrosis was manifested by disruption of nuclear architecture accompanied by an eosinophilic granular material in the tubular lumens. Cytoplasmic vacuolization of tubular epithelial cells and regenerating basophilic epithelial cells were usually more obvious in the peripheral areas of the cortex. In severely affected kidneys, eosinophilic proteinaceous material and cellular debris were present in tubular lumens in the medulla. Criteria for the diagnosis of nephropathy were as follows:

Minimal severity - approximately 10% or less of the renal cortex involved with the nephropathy

Mild severity - approximately 11% to 25% of the renal cortex involved with the nephropathy

Moderate severity - approximately 26 to 50% of the renal cortex involved with the nephropathy.

Nephropathy occurred in three male and seven female-A mice treated with 1,000 mg/kg clarithromycin alone (Table 12). Nephropathy was not diagnosed in male and female-A mice treated with AZT alone or with 250 or 500 mg/kg clarithromycin alone. Female-A mice appeared to be slightly more susceptible to the development of nephropathy than were male mice; however, the female-A mice were treated for approximately 30 days compared to approximately 20 days for the males. With combination therapy, the greatest incidence occurred in female-A mice treated with 1,000 mg/kg clarithromycin in combination with AZT. The nephropathy appears to be the result of treatment with clarithromycin as the incidence did not increase when combined with AZT.

TABLE 12
Histopathological Alterations in the Kidney in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations in Swiss (CD-1®) Mice

	Nephropathy[b]	
Dose[a]	Males	Females-A
0 + 0	0/10	0/20
0 + 250	0/10	0/20
0 + 500	0/10	0/20
0 + 1,000	3/10 (1 3)	7/20 (1 7)
200 + 0	0/10	0/20
200 + 250	0/10	2/20 (1 0)
200 + 500	0/10	0/20
200 + 1,000	1/10 (1 0)	5/20 (1 6)
400 + 0	0/10	0/20
400 + 250	0/10	0/20
400 + 500	1/10 (1 0)	1/20 (3 0)
400 + 1,000	1/10 (2 0)	5/20 (1 8)

[a] AZT + clarithromycin in mg/kg per day
[b] Number of animals with lesion/number of animals examined (mean severity for mice with lesion, grade 1=minimal, 2=mild, 3=moderate, 4=marked)

Lymph Node Lesions

Minimal to moderate depletion of lymphocytes occurred in the mesenteric and mandibular lymph nodes of two female-A mice treated with 1,000 mg/kg clarithromycin alone. Minimal cellular depletion was manifested by a reduction of approximately 10% of the lymphocyte population, and moderate depletion consisted of a reduction of approximately 26% to 50% of the lymphocyte population. Cytoplasmic vacuolization of reticuloendothelial cells accompanied the lymphoid depletion. Both mice with depletion of lymphocytes in lymph nodes had concurrent thymic atrophy and cellular depletion of lymphoid follicles in the spleen. Cellular depletion in lymph nodes was not diagnosed in male mice treated with clarithromycin alone at any dose level, in any of the female-A groups treated with 250 or 500 mg/kg clarithromycin alone, or in male or female-A mice treated with combinations of 200 or 400 mg/kg AZT and 250, 500, or 1,000 mg/kg clarithromycin.

Brain Lesions

A total of five mice had treatment-related lesions in the choroid plexus of the brain. Two female-A mice treated with 1,000 mg/kg clarithromycin alone, two female-A mice treated with 400 mg/kg AZT plus 1,000 mg/kg clarithromycin, and one male mouse treated with 400 mg/kg AZT plus 1,000 mg/kg clarithromycin had cytoplasmic vacuolization of cells of the choroid plexus. Severity grades ranged from minimal (involving up to

approximately 25% of the cells of the plexus) to mild (involving approximately 25% to 50% of the cells of the plexus), and in general, cytoplasmic vacuolization in cells of the choroid plexus occurred concurrently in mice that had cytoplasmic vacuolization in other tissues having cells with phagocytic capability (e.g., liver, spleen, lymph nodes). Macrophages have been demonstrated in the choroid plexus of mice (Sturrock, 1983, 1988). Cytoplasmic vacuolization of cells in the choroid plexus was not diagnosed in any male or female-A mice treated with AZT alone at any dose level, any male or female-A mice treated with 250 or 500 mg/kg clarithromycin alone, or any male mice treated with 1,000 mg/kg clarithromycin alone. This likely reflected the shorter duration of treatment for the male groups (approximately 20 days) compared to the female-A groups (approximately 30 days).

Heart Lesions

Treatment-related lesions occurred in the heart of three mice (one female-A mouse treated with 1,000 mg/kg clarithromycin alone and one male and one female-A mouse treated with 400 mg/kg AZT plus 1,000 mg/kg clarithromycin). The treatment-related heart lesions consisted of necrosis and hemorrhage of heart muscle, cytoplasmic vacuolization of valves, and degeneration of the atrium. The necrosis was manifested by disruption of the nuclear and cytoplasmic architecture accompanied by increased eosinophilia and a granular appearance. Vacuolization of the cytoplasm of cells in the heart valves resulted in a thickened to nodular appearance, and although the diagnosis of cytoplasmic vacuolization was documented in the valves, this cellular alteration was also present in cells of the endocardium and epicardium and around vessels extending into the heart muscle. Degeneration of the atrium was most obvious in the outer aspects of the wall of the atrium. The degenerative process consisted of cytoplasmic vacuolization of cells accompanied by edema and the deposition of granular eosinophilic material that may have been fibrin. The severity of the heart lesions ranged from minimal (involving up to approximetely 10% of the valves and the ventricular and atrial walls) to mild (involving approximetely 11% to 25% of the valves and the ventricular and atrial walls). Heart lesions did not occur in any of the male or female-A groups treated with AZT alone at any dose level, male or female-A groups treated with 250 or 500 mg/kg clarithromycin alone, or any of the lower dose combination groups.

Salivary Gland Lesions

Necrosis of the salivary gland occurred in two female-A mice treated with 1,000 mg/kg clarithromycin alone and in one male and one female-A mouse treated with 400 mg/kg AZT plus 1,000 mg/kg clarithromycin. The salivary gland was present with many of the mandibular lymph nodes; however, not all salivary glands were evaluated. The severity ranged from minimal (pyknosis and karyorrhexis of up to approximately 10% of glandular epithelial nuclei) to mild (pyknosis and karyorrhexis of approximately 11% to 25% of glandular epithelial nuclei). Distortion of the normal glandular pattern accompanied the cellular necrosis.

Nontreatment-Related Lesions

Microscopic lesions not attributed to the administration of either compound were seen in male and female-A mice. These lesions occurred with no distinct pattern in a variety of different treatment groups. One of the most common lesions consisted of hypertrophy of bone. This lesion consisted of a combination of cartilaginous metaplasia, fibrosis, and/or necrosis that occurred locally on thoracic vertebrae or ribs near their junction with the vertebrae. The lesions were examined microscopically as a result of nodules or a deformity observed on the vertebrae or ribs at necropsy, and they were attributed to mechanical irritation caused by the gavage procedure. Other incidental lesions were focal necrosis and focal inflammation of the liver, inflammation or ulceration of the skin or subcutaneous tissue, inflammation of skeletal muscle, focal inflammation and hyperplasia of the lung or mediastinum, renal cyst, focal inflammation of the kidney, mineralization or inflammation of the heart, hyperplasia of the iliac lymph node, hemorrhage in the mesentery, inflammation of the salivary gland, and dilatation of the renal pelvis.

Female-B Mice

Routine histopathological evaluations were not performed on female-B mice; however, gross lesions were processed and examined. Lesions in female-B mice believed to be related to treatment occurred only in the spleen and consisted of hematopoietic cell proliferation and hemosiderin pigmentation. For female-B groups treated with 200 mg/kg AZT plus 500 or 1,000 mg/kg clarithromycin and 400 mg/kg AZT plus 500 or 1,000 mg/kg clarithromycin, the respective incidences of hematopoietic cell proliferation of the spleen were one, one, one, and four. The degree of severity ranged from moderate to marked. Minimal hemosiderin pigmentation occurred in one, one, and three female-B mice treated, respectively, with 200 mg/kg AZT plus 1,000 mg/kg clarithromycin and 400 mg/kg AZT plus 500 or 1,000 mg/kg clarithromycin. The lesions in the spleen were believed to correspond with the anemia that occurred in these groups.

SPERM FUNCTION EVALUATION

Administration of 200 or 400 mg/kg AZT and 250, 500, or 1,000 mg/kg clarithromycin alone or in combination to male mice had no impact on male reproductive endpoints (Table D).

NATURAL DELIVERY DATA

Administration of 200 or 400 mg/kg AZT and 250, 500, or 1,000 mg/kgclartihromycin alone or in combination to female-B mice had no effect ($P \leq 0.05$) on the incidence of pregnancy (Table 13). Treatment with AZT alone or clarithromycin alone had no effect on the number of pregnant female-B mice that delivered litters. Combination therapy, however, had a distinct negative impact on the number of litters delivered. Slight declines in delivery

rates occurred in female-B groups treated with 200 mg/kg AZT plus 500 or 1,000 mg/kg clarithromycin, and a distinct dose-related decline in delivery rates occurred in groups treated with 400 mg/kg AZT and clarithromycin. Respective delivery rates for female-B groups treated with 400 mg/kg AZT alone and in combination with 250, 500, or 1,000 mg/kg clarithromycin were 100%, 42.8% ($P \leq 0.01$), 26.7% ($P \leq 0.01$), and 0.0% ($P \leq 0.01$).

TABLE 13
Occurrence of Pregnancy in Female-B Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations[a]

Dose[b]	No. Pregnant (%)	No. of Dams that Delivered (%)
0 + 0	15 (75 0)	14 (93 3)
200 + 0	14 (70 0)	14 (100 0)
400 + 0	16 (80 0)	16 (100 0)
0 + 250	15 (75 0)	15 (100 0)
0 + 500	12 (60 0)	11 (91 7)
0 + 1,000	14 (70 0)	14 (100 0)
200 + 250	15 (75 0)	15 (100 0)
200 + 500	14 (70 0)	8 (57 1)+
200 + 1,000	11 (55 0)	8 (72 7)
400 + 250	14 (70 0)	6 (42 8)**▲▲
400 + 500	15 (75 0)	4 (26 7)**▲▲
400 + 1,000	15 (75 0)	0 (0 0)**▲▲

[a] Number assigned to each group = 20
[b] AZT + clarithromycin in mg/kg per day
** Significantly different from the control group value ($P \leq 0.01$) by the Kruskal-Wallis test
+ Significantly different from the 200 + 0 group value ($P \leq 0.05$) by the Kruskal-Wallis test
▲▲ Significantly different from the 400 + 0 group value ($P \leq 0.01$) by the Kruskal-Wallis test

Combination therapy tended to increase the duration of gestation. The duration of gestation (Table 14) was increased in female-B groups treated with 200 mg/kg AZT plus 500 or 1,000 mg/kg clarithromycin, as well as the groups treated with 400 mg/kg AZT plus 250 or 500 mg/kg clarithromycin. Respective mean durations of gestation for the above groups were 20.6 days, 20.8 days ($P \leq 0.05$), 21.0 days, and 20.5 days compared to 20.0 days for the controls. Administration of AZT or clarithromycin alone did not alter the duration of gestation.

For the dams treated with 400 mg/kg AZT alone, 18.8% had stillborn pups and 14.3% of dams treated with 1,000 mg/kg clarithromycin alone had stillborn pups (Table 14). For the female-B groups treated with 200 mg/kg AZT plus 250, 500, or 1,000 mg/kg clarithromycin, the percentages of dams with stillborn pups were, respectively, 13.3%, 25.0%, and 25.0%. There were no litters delivered in the group treated with 400 mg/kg AZT plus 1,000 mg/kg clarithromycin, and stillborn pups did not occur in groups treated with 250 or 500 mg/kg

clarithromycin alone, 200 mg/kg AZT alone, or the groups treated with 400 mg/kg AZT plus 250 or 500 mg/kg clarithromycin.

Although not statistically significant ($P \leq 0.05$), combination therapy caused declines in litter size (Table 14). For the groups treated with 200 mg/kg AZT plus 250, 500, or 1,000 mg/kg clarithromycin, respective mean liveborn litter sizes were 10.5, 8.6, and 6.1 pups per litter. For the groups treated with 400 mg/kg AZT plus 250 or 500 mg/kg clarithromycin, mean liveborn litter sizes were 5.2 and 6.8 pups per litter. There were no litters delivered in the group treated with 400 mg/kg AZT plus 1,000 mg/kg clarithromycin.

Prominent declines in viability indices (total number of pups surviving to postnatal day 4/total number of liveborn pups on day 0 postpartum × 100) occurred in female-B groups treated with the higher dose combinations (Table 14). In general, the mean number of surviving pups per litter on day 4 paralleled the treatment-related declines in the viability indices. The viability index in the group treated with 400 mg/kg AZT alone was slightly reduced (93.4%, $P \leq 0.05$). Further reductions occurred with combination therapy. For the group treated with 200 mg/kg AZT plus 1,000 mg/kg clarithromycin, the viability index was 62.8% ($P \leq 0.01$). Respective indices for groups treated with 400 mg/kg AZT plus 250 or 500 mg/kg clarithromycin were 33.3% ($P \leq 0.01$) and 81.5% ($P \leq 0.01$).

Administration of AZT alone or clarithromycin alone did not result in reduced pup weights. Pup weights per litter (Figure 11) were reduced for groups treated with 200 mg/kg AZT plus 250, 500, or 1,000 mg/kg clarithromycin, with 400 mg/kg AZT alone, and with 400 mg/kg AZT plus 250 or 500 mg/kg clarithromycin.

TABLE 14
Summary of Significant Natural Delivery Litter Data for Female-B Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations

Dose[a]	Duration of Gestation (Days)	Dams with Stillborn Pups (%)[b]	Dams with All Pups Dying on Days 0-4 (%)[b]	Mean Liveborn Pups per Litter	Mean Surviving Pups per Litter Day 4	Viability Index (%)[c]
0 + 0	20 0	0 (0 0)	0 (0 0)	12 0	11 8	98 2
200 + 0	20 2	0 (0 0)	0 (0 0)	11 1	11 1	99 4
400 + 0	20 1	3 (18 8)	0 (0 0)	12 2	11 4	93 4*
0 + 250	20 0	0 (0 0)	0 (0 0)	12 2	12 1	98 9
0 + 500	19 9	0 (0 0)	0 (0 0)	12 0	12 0	100 0
0 + 1,000	20 1	2 (14 3)	0 (0 0)[d]	10 3	10 0	97 6
200 + 250	20 3	2 (13 3)	0 (0 0)[d]	10 5	10 2	98 6
200 + 500	20 6	2 (25 0)	0 (0 0)[d]	8 6	7 3	91 7+
200 + 1000	20 8*	2 (25 0)	1 (12 5)	6 1	3 8**++	62 8**++
400 + 250	21 0	0 (0 0)	2 (33 3)[d]	5 2	1 0**▲▲	33 3**▲▲
400 + 500	20 5	0 (0 0)	0 (0 0)	6 8	5 5	81 5**
400 + 1,000[e]						

[a] AZT + clarithromycin in mg/kg per day
[b] % of dams that delivered litters
[c] Number of live pups on day 4/number of liveborn pups on day 0 × 100
[d] Excludes values for litter found drowned
[e] No litters delivered
* Significantly different from control group (P≤0 05) by Cochran-Armitage and Fisher's exact tests
** Significantly different from control group (P≤0 01) by Cochran-Armitage and Fisher's exact tests
++ Significantly different from 200 + 0 group (P≤0 01) by Cochran-Armitage and Fisher's exact tests
▲▲ Significantly different from 400 + 0 group (P≤0 01) by Cochran-Armitage and Fisher's exact tests

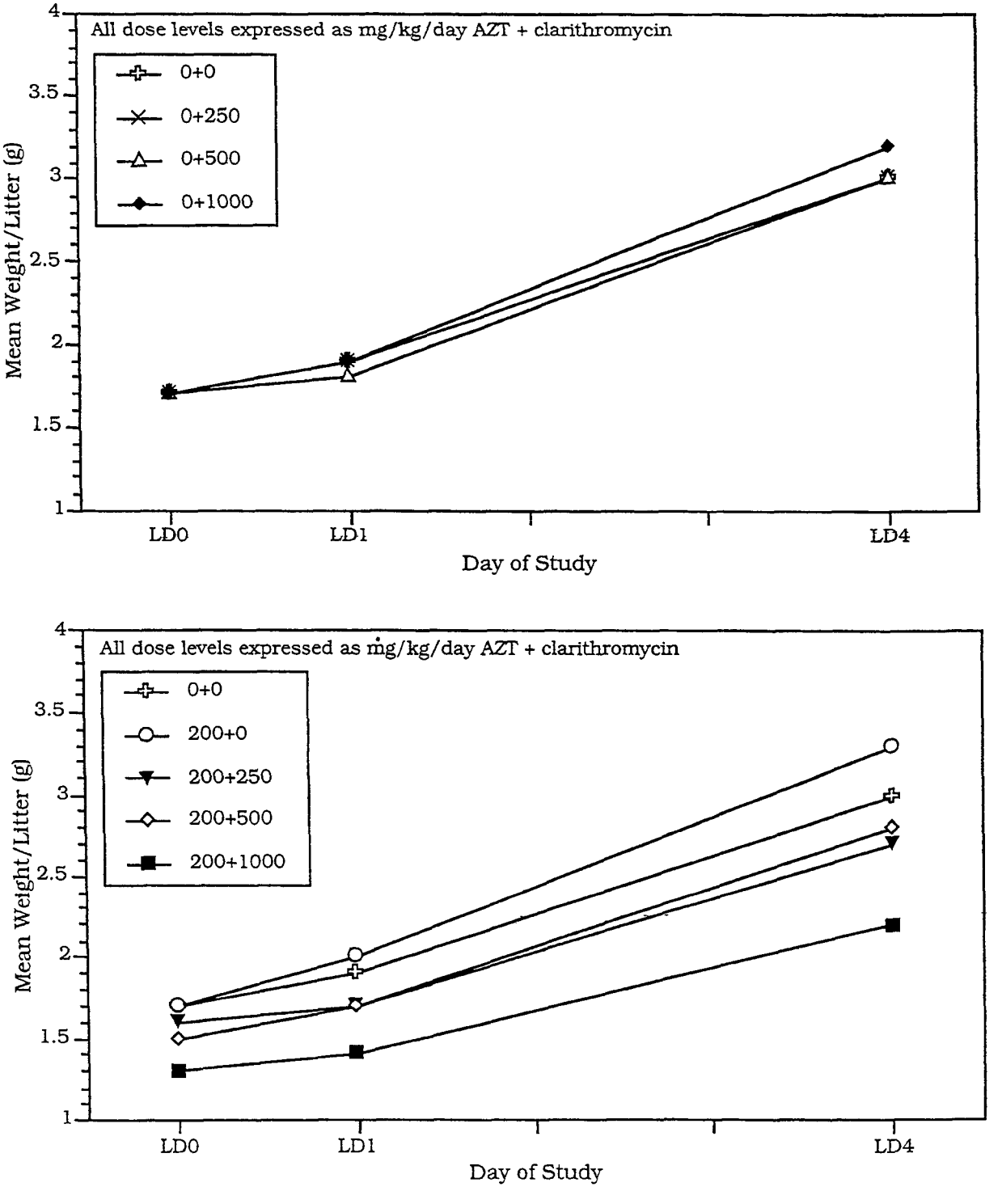


FIGURE 11
Mean Body Weights per Litter of Female-B Pups in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations in Swiss (CD-1®) Mice
(LD=Lactation Day)

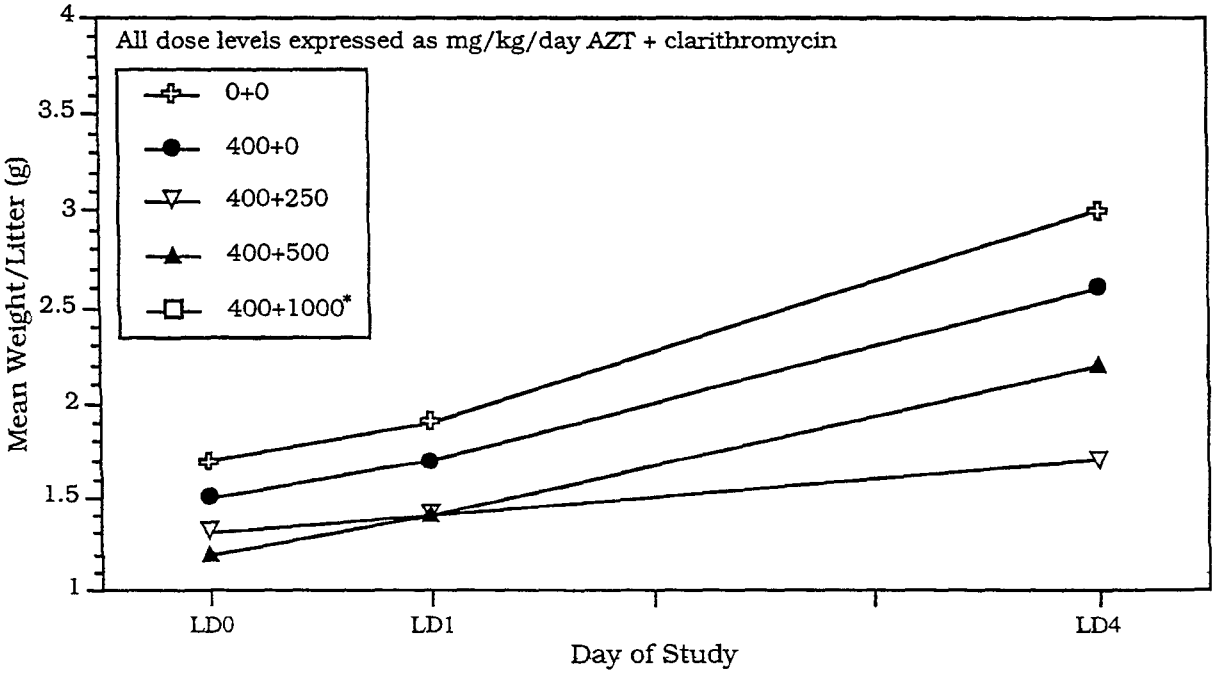


FIGURE 11 (continued)
Mean Body Weights per Litter of Female-B Pups in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations in Swiss (CD-1®) Mice
(LD=Lactation Day; * No litters were delivered)

CAESAREAN SECTION DATA

Slight declines in pregnancy rates (Table 15) occurred in female-A mice treated with 400 mg/kg AZT alone and 1,000 mg/kg clarithromycin alone. Compared to the control group, with a pregnancy rate of 100%, female-A groups treated with 200 or 400 mg/kg AZT had pregnancy rates of 80% and 75% ($P \leq 0.05$), respectively. For the groups treated with 250, 500, or 1,000 mg/kg clarithromycin, respective pregnancy rates were 95%, 90%, and 70% ($P \leq 0.05$). Further declines in pregnancy rates occurred with combination therapy, and the declines appeared to parallel increasing doses of AZT and clarithromycin. For the groups treated with 200 mg/kg AZT plus 250, 500, or 1,000 mg/kg clarithromycin, respective pregnancy rates were 95%, 80%, and 45% ($P \leq 0.01$). For the groups treated with 400 mg/kg AZT plus 250, 500, or 1,000 mg/kg clarithromycin, respective pregnancy rates were 65% ($P \leq 0.01$), 20% ($P \leq 0.01$), and 10% ($P \leq 0.01$).

Litter size and the number of live fetuses per litter were altered by administration of AZT alone and clarithromycin alone (Table 16). Administration of AZT alone also caused a slight decline in litter size and the number of live fetuses per litter. Compared to the control group, which had 10.9 live fetuses per litter, groups treated with 200 or 400 mg/kg AZT had 8.2 and 4.3 ($P \leq 0.01$) live fetuses per litter, respectively. For groups treated with 250, 500, or 1,000 mg/kg clarithromycin, there were 11.2, 10.9, and 9.8 live fetuses per litter. Combination therapy caused

TABLE 15
Occurrence of Pregnancy in Female-A Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations

Dose[a]	No. Assigned	No. Pregnant (%)
0 + 0	20	20 (100 0)
200 + 0	20	16 (80 0)
400 + 0	20	15 (75 0)*
0 + 250	20	19 (95 0)
0 + 500	20	18 (90 0)
0 + 1,000	20	14 (70 0)*
200 + 250	20	19 (95 0)
200 + 500	20	16 (80 0)
200 + 1,000	20	9 (45 0)**+
400 + 250	20	13 (65 0)**
400 + 500	20	4 (20 0)**▲▲
400 + 1,000	20	2 (10 0)**▲▲

[a] AZT + clarithromycin in mg/kg per day
* Significantly different from control group ($P \leq 0$ 05) by Cochran-Armitage and Fisher's exact tests
** Significantly different from control group ($P \leq 0$ 01) by Cochran-Armitage and Fisher's exact tests
\+ Significantly different from 200 + 0 group ($P \leq 0$ 05) by Cochran-Armitage and Fisher's exact tests
▲▲ Significantly different from 400 + 0 group ($P \leq 0$ 01) by Cochran-Armitage and Fisher's exact tests

further declines in live litter size. For the groups treated with 200 mg/kg AZT plus 250, 500, or 1,000 mg/kg clarithromycin, there were 7.8, 6.4 ($P \leq 0.01$), and 6.4 ($P \leq 0.05$) live fetuses per litter. For those treated with 400 mg/kg AZT plus 250, 500, or 1,000 mg/kg clarithromycin, there were 2.3 ($P \leq 0.01$), 0.0 ($P \leq 0.05$), and 2.0 live fetuses per litter, respectively. Mortality in female-A dams did not influence the number of live fetuses per litter. Administration of AZT alone also increased the mean number of resorptions per litter (Table 16). The control group averaged 0.7 resorptions per litter, female-A mice treated with 200 or 400 mg/kg AZT had 3.6 ($P \leq 0.01$) and 5.1 ($P \leq 0.01$) resorptions per litter. Administration of 250, 500, or 1,000 mg/kg clarithromycin alone did not alter ($P \leq 0.05$) the mean number of resorptions per litter. Combination therapy increased the mean number of resorptions per litter. For the groups treated with 200 mg/kg AZT plus 250, 500, or 1,000 mg/kg clarithromycin, the mean number of resorptions per litter were 4.0 ($P \leq 0.01$), 4.7 ($P \leq 0.01$), and 6.2 ($P \leq 0.01$), respectively. For those treated with 400 mg/kg AZT and the same dosages of clarithromycin, the respective mean number of resorptions were 8.2 ($P \leq 0.01$), 11.0 ($P \leq 0.05$), and 7.0 ($P \leq 0.05$) per litter.

TABLE 16
Summary of Significant Caesarean-Sectioning Litter Data for Female-A Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations

	Litter Size	Live Fetuses		Dead Fetuses		Resorptions	
Dose[a]	Mean ± SD	No.	Mean ± SD	No.	Mean ± SD	No.	Mean ± SD
0 + 0	10 9 ± 3 1	197	10 9 ± 3 1	0	0 0 ± 0 0	13	0 7 ± 0 7
200 + 0	8 2 ± 3 0	131	8 2 ± 3 0	0	0 0 ± 0 0	58	3 6 ± 2 9**
400 + 0	4 3 ± 3 2**	64	4 3 ± 3 2**	0	0 0 ± 0 0	77	5 1 ± 3 4**
0 + 250	11 4 ± 1 9	180	11 2 ± 2 1	3	0 2 ± 0 5	17	1 1 ± 1 3
0 + 500	11 0 ± 2 1	163	10 9 ± 2 1	2	0 1 ± 0 4	16	1 1 ± 0 9
0 + 1,000	9 8 ± 4 4	117	9 8 ± 4 4	0	0 0 ± 0 0	23	1 9 ± 3 0
200 + 250	7 9 ± 5 1	141	7 8 ± 5 2	2	0 1 ± 0 3	73	4 0 ± 3 4**
200 + 500	6 6 ± 3 4**	102	6 4 ± 3 4**	4	0 2 ± 0 4	75	4 7 ± 2 0**
200 + 1,000	6 8 ± 4 1*	58	6 4 ± 3 9*	3	0 3 ± 1 0	56	6 2 ± 2 9**
400 + 250	2 5 ± 4 4**	30	2 3 ± 4 3**	3	0 2 ± 0 4	107	8 2 ± 3 1**
400 + 500	0 0 ± 0 0*	0	0 0 ± 0 0*	0	0 0 ± 0 0	44	11 0 ± 3 8*
400 + 1,000	2 5 ± 3 5	4	2 0 ± 2 8	1	0 5 ± 0 7*	14	7 0 ± 1 4*

[a] AZT + clarithromycin in mg/kg per day
* Significantly different from control group ($P \leq 0$ 05) by Cochran-Armitage and Fisher's exact tests
** Significantly different from control group ($P \leq 0$ 01) by Cochran-Armitage and Fisher's exact tests

Fetal weights (Table 17) were reduced in all groups administered AZT either alone or in combination with clarithromycin. Although not statistically significant ($P \leq 0.05$) when compared to the mean fetal weight in the control group (1.38 grams), groups treated with 200 or 400 mg/kg AZT had mean fetal weights of 1.24 and 1.17 grams, respectively. Significant ($P \leq 0.05$) declines in mean fetal weights did not occur in groups treated with clarithromycin alone. For the groups treated with 200 mg/kg AZT plus 250, 500, or 1,000 mg/kg clarithromycin, mean fetal weights were 1.04 grams ($P \leq 0.05$), 0.92 grams ($P \leq 0.01$), and 0.90 grams ($P \leq 0.01$), respectively. For the groups treated with 400 mg/kg AZT plus 250 or 1,000 mg/kg clarithromycin, respective mean fetal weights were 1.03 and 0.92 grams. There were no pups available for weighing in the group treated with 400 mg/kg AZT plus 500 mg/kg clarithromycin.

TABLE 17
Body Weights of Fetuses from Female-A Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations

Dose[a]	Mean Fetal Weight (grams)
0 + 0	1 38
200 + 0	1 24
400 + 0	1 17
0 + 250	1 25
0 + 500	1 28
0 + 1,000	1 30
200 + 250	1 04*
200 + 500	0 92**
200 + 1,000	0 90**
400 + 250	1 03
400 + 500	—
400 + 1,000	0 92

[a] AZT + clarithromycin in mg/kg per day
— No fetuses were present
* Significantly different from control group ($P \leq 0$ 05) by Dunnett's or Dunn's test
** Significantly different from control group ($P \leq 0$ 01) by Dunnett's or Dunn's test

GROSS EXTERNAL ALTERATIONS

A slight increase in the number of litters with fetuses with hydramnios occurred in the groups treated with 200 mg/kg AZT plus 500 or 1,000 mg/kg clarithromycin (Table 18). The fetal incidence was significant ($P \leq 0.01$) in the group treated with 200 mg/kg AZT plus 1,000 mg/kg clarithromycin. Similar increases probably did not occur in groups treated with 400 mg/kg AZT plus clarithromycin because of the reduced number of fetuses in these dose groups. There were no litters available for evaluation in the group treated with 400 mg/kg AZT plus 500 mg/kg clarithromycin and only one litter was available for evaluation in the group treated with 400 mg/kg AZT plus 1,000 mg/kg clarithromycin.

Other alterations included clubfoot of the hindlimbs, dark focus on the head, cranioschisis, hematoma on the head, pale body, dark focus on the body, spina bifida occulta, dark focus on the foot, dark eye, fetuses that were too small or fragile to sex or examine, and a nonspecified hematoma. These alterations were not believed to be related to treatment because the observation occurred in a control group litter, the observation may have been related to trauma, and/or the observation may reflect the mistiming of a single pregnancy.

TABLE 18
Summary of Fetal Gross External Alterations in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations in Swiss (CD-1®) Mice

Observation	Dose[a]	Litter Incidence[b]	Fetal Incidence[c]
Hydramnios	0 + 0	1 (5 6)	13 (6 6)
	200 + 0	2 (12 5)	13 (9 9)
	400 + 0	0 (0 0)	0 (0 0)*
	0 + 250	0 (0 0)	0 (0 0)**
	0 + 500	1 (6 7)	13 (8 0)
	0 + 1,000	0 (0 0)	0 (0 0)**
	200 + 250	0 (0 0)	0 (0 0)**++
	200 + 500	4 (26 7)	14 (14 0)
	200 + 1,000	2 (25 0)	16 (27 6)**++
	400 + 250	1 (14 3)	2 (6 7)
Clubfoot hindlimbs	0 + 0	6 (33 3)	6 (3 0)
	200 + 0	0 (0 0)*	0 (0 0)
	400 + 0	1 (8 3)	1 (1 6)
	0 + 250	3 (18 8)	3 (1 7)
	0 + 500	1 (6 7)	2 (1 2)
	0 + 1,000	1 (9 1)	1 (0 8)
	200 + 250	0 (0 0)*	0 (0 0)
	200 + 500	0 (0 0)*	0 (0 0)
Head, dark focus	400 + 1,000	1 (100 0)[d]	1 (25 0)
Head, cranioschisis	200 + 500	1 (6 7)	1 (1 0)
Head, hematoma	200 + 0	1 (6 2)	1 (0 8)
Body, pale	200 + 500	1 (6 7)	1 (1 0)
	400 + 1,000	1 (100 0)[d]	4 (100 0)
Body, dark focus	200 + 500	1 (6 7)	1 (1 0)
Body, spina bifida occulta	200 + 500	1 (6 7)	1 (1 0)
Too small and fragile to sex or examine	0 + 0	1 (5 6)	13 (6 6)
	200 + 0	1 (6 2)	12 (9 2)
	400 + 0	0 (0 0)	0 (0 0)*
	0 + 250	1 (6 2)	13 (7 2)
	0 + 500	1 (6 7)	13 (8 0)
	0 + 1,000	0 (0 0)	0 (0 0)**
	200 + 250	1 (6 2)	13 (9 2)
	200 + 500	0 (0 0)	0 (0 0)**++
	200 + 1,000	0 (0 0)	0 (0 0)+
Right foot, dark focus	0 + 1,000	1 (9 1)	1 (0 8)
Right eye dark	0 + 250	1 (6 2)	1 (0 6)
Hematoma	0 + 0	1 (5 6)	2 (1 0)

[a] AZT + clarithromycin in mg/kg per day
[b] Data are presented as number of litters with the observation followed by percent (number of litters with the observation/number of litters evaluated)
[c] Data are presented as number of fetuses with the observation followed by percent (number of fetuses with the observation/number of fetuses evaluated, excludes dead fetuses)
[d] Excluded from statistical analyses as there was only one litter in the group
* Significantly different from control group ($P \leq 0.05$) by Dunnett's or Dunn's test
** Significantly different from control group ($P \leq 0.01$) by Dunnett's or Dunn's test
+ Significantly different from 200 + 0 group ($P \leq 0.05$) by Dunnett's or Dunn's test
++ Significantly different from 200 + 0 group ($P \leq 0.01$) by Dunnett's or Dunn's test

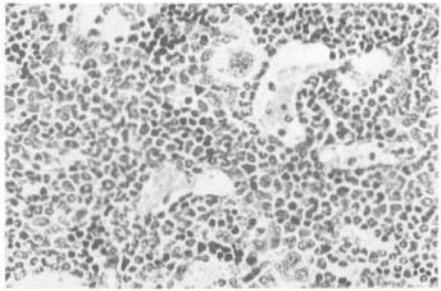


PLATE 1
Bone marrow from a control female-A mouse. (×400)

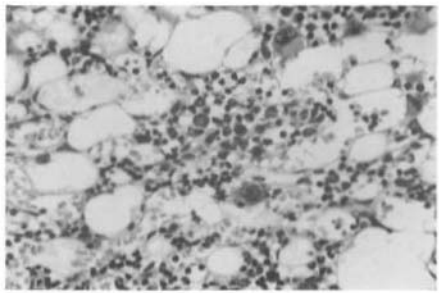


PLATE 2
Bone marrow from a female-A mouse treated with 400 mg/kg AZT + 500 mg/kg clarithromycin showing cellular depletion. (×400)

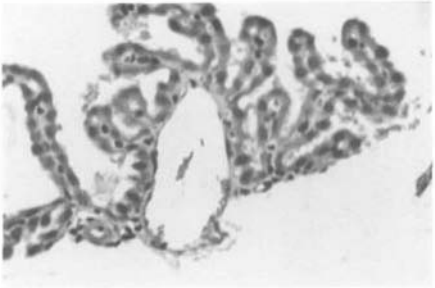


PLATE 3
Choroid plexus in the brain of a control female-A mouse. (×400)

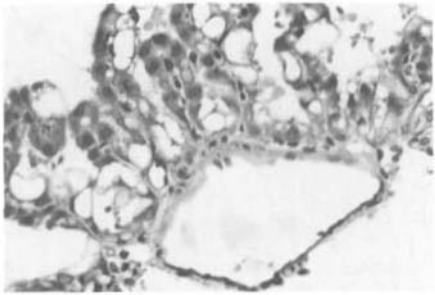


PLATE 4
Choroid plexus in the brain of a female-A mouse treated with 1,250 mg/kg clarithromycin showing cytoplasmic vacuoles in cells of the plexus. (×400)

DISCUSSION AND CONCLUSIONS

Two reproductive, developmental, and general toxicity studies were conducted in Swiss (CD-1®) mice treated with AZT alone, clarithromycin alone, and combinations of AZT and clarithromycin. In the first study, the dosages of AZT were 200 and 400 mg/kg per day, and the dosages of clarithromycin were 500, 1,250, and 2,500 mg/kg per day. The clarithromycin contained numerous excipients included by the manufacturer in the preparation of these antibiotic tablets for human therapeutic use. The dose formulations were administered by oral gavage for approximately 20 days to male mice, approximately 30 days to female-A mice, and approximately 10 days to female-B mice. Excessive mortality occurred in male and female groups treated with 1,250 or 2,500 mg/kg clarithromycin alone or in combination with AZT. The mortality was attributed to toxicity in multiple vital organs and localized complications in the stomach associated with chronic dilatation from the large volume of thick gavaged material. The numerous excipients likely contributed to the viscosity of the clarithromycin formulations, resulting in chronic dilatation of the stomach. The local antibacterial action of clarithromycin apparently altered the normal flora in the stomach lumen, resulting in a fungal overgrowth and inflammatory lesions. In addition to the high mortality rate associated with the higher dosages of clarithromycin, cytoplasmic vacuolization of phagocytic cells occurred in many tissues (brain, bone marrow, spleen, liver, kidney, thymus, lymph nodes). An example of this lesion in the choroid plexus of the brain is illustrated in Plates 3 and 4. Groups treated with AZT and clarithromycin combinations developed severe hematopoietic toxicity with bone marrow depression, anemia, and high mortality rates. Reduced fertility also occurred in groups treated with higher doses of clarithromycin alone or in combination with AZT.

Because of excessive mortality in the study conducted with unpurified clarithromycin, a second study was conducted using purified clarithromycin at lower dosage levels. Male and female Swiss (CD-1®) mice were treated by gavage with AZT alone (200 or 400 mg/kg per day), clarithromycin alone (250, 500, or 1,000 mg/kg per day), and combinations of AZT and clarithromycin. As in the first study, male mice were treated for approximately 20 days, female-A mice for approximately 30 days, and female-B mice for approximately 10 days.

Treatment with AZT alone at 400 mg/kg resulted in higher plasma concentrations in male mice treated for approximately 20 days than in pregnant female-A mice treated for approximately 30 days. This difference may be a reflection of the physiological state of pregnancy. Administration of 400 mg/kg AZT in combination with 250, 500, or 1,000 mg/kg clarithromycin resulted in peak plasma concentrations of AZT decreasing in both sexes as the oral dose of clarithromycin increased. The patterns of β-D-glucuronide of AZT (GAZT) plasma concentrations were similar to the AZT plasma concentrations. The 3′-amino-3′-deoxythymidine (AMT)

concentrations show some clarithromycin dose-related pattern in the female-A mice but not in the males. Drug interactions similar to this have been reported previously in AIDS patients treated with clarithromycin and AZT (Polis *et al.*, 1997). The authors postulated that the lower plasma levels of AZT may be related to altered absorption of AZT and recommended that administration of AZT and clarithromycin to AIDS patients be separated by at least 2 hours. Results of this study indicate that the metabolism and disposition of AZT in pregnant female mice treated with AZT/clarithromycin combinations for approximately 30 days may be slower than in male mice treated for approximately 20 days. This could also be a reflection of altered absorption of AZT secondary to clarithromycin-induced changes in gastrointestinal motility. The physiological state of pregnancy as well as the differences in the duration of treatment could also influence the metabolism of AZT.

There was no marked dose- or time-related trend for plasma concentrations of clarithromycin from 30 to 240 minutes after oral administration, either alone or in combination with 400 mg/kg AZT. However, the plasma concentrations of clarithromycin in pregnant females were several-fold higher than in the males at all time points included in this study, indicating slower elimination of clarithromycin in pregnant females than in males. One of the reasons for higher concentrations of clarithromycin in pregnant females may be due to longer duration of treatment of females (30 days) compared to males (20 days).

Treatment with AZT alone resulted in measurable evidence of hematopoietic toxicity manifested by mild decreases in red blood cell, hemoglobin, and hematocrit values and mild to moderate increases in mean cell volume (MCV) and red cell distribution width values in both male and female mice. Hematopoietic cell proliferation of the spleen and slight hemosiderosis of the spleen accompanied the mild alterations in erythrocyte parameters. A minor degree of cytoplasmic alteration believed to represent glycogen depletion occurred in hepatocytes in male and female-A mice, and a slight reduction in body weight gain occurred in the high-dose female-A group. Mortality and significant clinical alterations did not accompany the slight hematopoietic toxicity that occurred in male and female mice treated with AZT.

The macrocytosis that accompanied the slight anemia was related to dose and duration of treatment, as the severity was greatest in female-A mice treated for approximately 30 days when compared to the male mice treated for approximately 20 days. Hematological alterations in female-B mice treated with AZT alone for approximately 10 days were limited to mild elevations in MCV values. Reticulocytosis did not accompany the macrocytosis in any of the male or female treatment groups. The mild macrocytic anemia that occurred with AZT treatment is compatible with the anemia previously reported in mice (Thompson *et al.*, 1991). Macrocytic anemia is a common observation in humans treated with AZT (Richman *et al.*, 1987; Snower and Weil, 1993). The exact mechanism

of the erythrocyte macrocytosis is unclear but it likely reflects inhibition of DNA synthesis in erythroid precursors. AZT is directly toxic to erythroid blast forming units (BFU-E) and erythroid colony forming units (CFU-E) *in vitro* at high concentrations and is antiproliferative in CFU-E at lower concentrations (Gogu *et al.*, 1995). In *in vivo* studies, AZT has been shown to increase splenic and bone marrow BFU-E in mice and to increase the sensitivity of both splenic and bone marrow BFU-E to erythropoietin (Chow *et al.*, 1991); both effects occur in the absence of an appreciable regenerative response (reticulocytosis), suggesting a maturation block in the erythroid series due to a block in terminal differentiation. AZT has since been shown to down-regulate the erythropoietin receptor in CFU-E and inhibit erythropoietin receptor-mediated signal transduction (Gogu *et al.*, 1995). These mechanisms could inhibit CFU-E proliferation and possibly affect erythropoietin-regulated maturation (Bick, 1993). The absence of reticulocytosis in the presence of a macrocytic anemia subsequent to the administration of AZT in this study is compatible with depression of the erythroid precursors due to impaired DNA synthesis.

Administration of 250 or 500 mg/kg clarithromycin alone did not result in significant toxicity in male or female mice. Administration of 1,000 mg/kg clarithromycin alone resulted in a low incidence of toxicity in male and female mice involving multiple organs, including liver, spleen, bone marrow, kidney, heart, brain, thymus, lymph nodes, and salivary gland. The low incidence of these lesions is likely the result of individual susceptibility to the effects of clarithromycin, as the majority of the mice in these groups did not have lesions. Vacuolization of phagocytic cells occurred in many of these tissues and was especially evident in the spleen, bone marrow, liver, and choroid plexus of the brain. Phagocytic capabilities of the reticuloendothelial cells in lymph nodes, spleen, liver, and bone marrow are easily visualized by the presence of hemosiderin in these tissues. Macrophages with phagocytic capabilities have been demonstrated on the surface of the choroid plexus in the brain of mice (Sturrock, 1983, 1988). Cellular uptake of clarithromycin has been demonstrated *in vitro* in neutrophils and macrophages (Baradell *et al.*, 1993), but how large amounts of clarithromycin affect macrophage morphology and other cells with phagocytic capabilities *in vivo* has not been described.

Administration of AZT in combination with clarithromycin resulted in hematopoietic toxicity in male and female-A mice, and the severity of the anemia was far greater than that induced by AZT alone. The anemia was accompanied by bone marrow depletion and hematopoietic cell proliferation and hemosiderosis of the spleen. The anemia was macrocytic in the majority of the treatment groups, but progressed to a microcytic anemia in the highest dose female-A group. Clarithromycin has been previously associated with a nonregenerative normocytic to microcytic anemia (Guay *et al.*, 1993). Reticulocytopenia and sporadic thrombocytosis were also considered to be treatment-related manifestations of hematopoietic toxicity. In general, combination therapy did not appear to enhance toxicity in other tissues as the morphological alterations and the incidence and severity were similar

to those induced by clarithromycin alone. Toxicity, in general, was more severe in female-A mice treated for approximately 30 days than in male mice treated for approximately 20 days. Toxicity was least evident in female-B mice treated for approximately 10 days. Toxicological basis for increases in hematological toxicity of AZT+clarithromycin combinations is not known. However, increased hematological toxicity with increasing doses of clarithromycin in combination with a fixed dose of AZT in female-A mice may be due to clarithromycin dose-related increases in plasma concentrations of AZT and clarithromycin and longer duration of treatment than in the males.

Regarding reproductive toxicity, treatment with AZT alone resulted in a slight decline in pregnancy rate, reduced live litter size, increased numbers of resorptions, and a slight decline in total weight per litter. These alterations have been previously reported in mice treated with AZT alone (NIEHS, 1999). Treatment with clarithromycin alone resulted in diminished fertility with a reduction in pregnancy rate, reduction in the number of litters delivered and a reduced litter size. Clarithromycin alone did not cause an increase in gross external alterations (cleft palate) as previously reported (Abbott Laboratories, 1998). Fertility was further reduced in groups treated with combinations of AZT and clarithromycin. Combination therapy resulted in reduced pregnancy rates, reduced live litter size, increased numbers of resorptions and declines in fetal and pup weights per litter. A decreased number of pups surviving to postnatal day 4 also occurred. However, most increases in reproductive and developmental toxic effects in female mice were observed at the combination dose levels that caused increased maternal toxicity as measured by hematological parameters and body weights.

CONCLUSIONS

Two reproductive, developmental, and general toxicity studies were conducted in Swiss (CD-1®) mice treated with AZT (200 or 400 mg/kg per day) and clarithromycin. In the first study, using unpurified clarithromycin formulations (prepared from tablets for human use and containing excipients included by the manufacturer) of 500, 1,250, or 2,500 mg/kg per day, excessive mortality occcurred due to multiple organ toxicity and stomach complications secondary to the large volume of gavaged material and fungal infections. In the second study, mice were treated with AZT and 250, 500, or 1,000 mg/kg purified clarithromycin.

Administration of AZT alone resulted in mild hematopoietic toxicity manifested primarily by a slight macrocytic anemia. Administration of 250 or 500 mg/kg clarithromycin alone did not result in significant toxicity in male or female mice. Administration of 1,000 mg/kg clarithromycin resulted in a low incidence of toxicity (cytoplasmic vacuolization of phagocytic cells) in multiple organs.

Administration of AZT (200 or 400 mg/kg) in combination with clarithromycin (250, 500, or 1000 mg/kg) resulted in exacerbation of the hematopoietic toxicity induced by AZT alone. The severity of the anemia with combination therapy was far greater than that induced by AZT alone, and the anemia was accompanied by cellular depletion of the bone marrow and hematopoietic cell proliferation in the spleen. The anemia was macrocytic in the majority of the combination therapy groups but progressed to a microcytic anemia in the female-A group treated with the highest dose of AZT and clarithromycin. Other manifestations of hematopoietic toxicity resulting from combination therapy consisted of reticulocytopenia and thrombocytosis.

Regarding reproductive toxicity, treatment with AZT alone resulted in a slight decline in pregnancy rate, reduced live litter size, increased numbers of resorptions, and a slight decline in total weight per litter. Treatment with clarithromycin alone resulted in diminished fertility with a reduction in pregnancy rate, reduction in the number of litters delivered and reduced litter size. Fertility was further reduced in groups treated with combinations of AZT and clarithromycin. Combination therapy resulted in reduced pregnancy rates, reduced live litter size, increased numbers of resorptions, and declines in fetal and pup weights per litter. A decreased number of pups surviving to postnatal day 4 also occurred. However, the increases in reproductive and developmental toxicity of the therapeutic combinations may be due to increased maternal toxicity. These results indicate that AZT and clarithromycin combinations have the potential to markedly increase the hematopoietic toxicity of either therapy alone.

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APPENDIX A
BODY WEIGHTS AND ORGAN WEIGHTS

TABLE A1
Summary of Body Weights for Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations

Male Mice

	Mean Body Weights[b] on Study Day						
Dose[a]	3	5	9	13	17	21	23
0 + 0	36 8 ± 1 7	36 7 ± 1 9	36 8 ± 1 9	36 3 ± 2 0	36 3 ± 2 0	36 6 ± 2 1	36 4 ± 1 8
200 + 0	37 2 ± 2 1	37 0 ± 2 3	37 1 ± 2 0	37 0 ± 1 9	37 1 ± 2 1	37 2 ± 1 9	37 0 ± 2 0
400 + 0	37 5 ± 1 3	36 9 ± 1 1	37 3 ± 1 6	36 9 ± 2 0	36 7 ± 2 6	37 3 ± 2 4	37 7 ± 2 3 (9)
0 + 250	37 1 ± 1 4	36 9 ± 1 4	37 3 ± 1 4	36 8 ± 2 1	37 2 ± 1 8	37 2 ± 1 5	37 9 ± 1 3
0 + 500	37 5 ± 1 8	37 3 ± 1 2	37 5 ± 1 4	37 3 ± 1 6	36 7 ± 1 5	37 8 ± 1 4	38 3 ± 1 3
0 + 1,000	37 1 ± 2 0	37 7 ± 2 0	37 6 ± 2 1	36 7 ± 1 4	36 3 ± 3 1	37 6 ± 2 2	37 7 ± 2 3
200 + 250	37 0 ± 1 0	37 3 ± 1 0	37 3 ± 1 4	37 1 ± 1 4	37 1 ± 1 2	36 6 ± 1 7	37 6 ± 1 8
200 + 500	36 7 ± 1 4	37 5 ± 1 6	37 6 ± 1 0	37 2 ± 1 7	37 2 ± 1 7	37 4 ± 1 4	38 2 ± 2 0
200 + 1,000	36 1 ± 3 2	38 0 ± 1 8	37 8 ± 1 9	36 9 ± 1 4	36 6 ± 1 2	36 2 ± 1 5	36 7 ± 1 8
400 + 250	36 9 ± 1 6	37 2 ± 1 5	37 7 ± 1 5	37 5 ± 1 4	37 3 ± 1 3	37 8 ± 0 8	38 3 ± 1 3
400 + 500	37 4 ± 1 3	37 6 ± 1 2	38 3 ± 1 6	37 8 ± 1 6	37 6 ± 1 6	36 9 ± 2 2	37 8 ± 1 6
400 + 1,000	37 4 ± 2 0	38 1 ± 1 9	38 7 ± 2 3	36 7 ± 2 2	36 1 ± 2 7	36 7 ± 3 0	37 2 ± 3 6

[a] AZT + clarithromycin in mg/kg per day
[b] Weight (grams) expressed as group mean ± standard deviation, n = 10 unless otherwise noted in parentheses

TABLE A1
Summary of Body Weights for Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations

Female-A Mice

	Mean Body Weights[b] on Study Day			
Dose[a]	**0**	**4**	**12**	**16**
0 + 0	29 4 ± 1 6	29 7 ± 1 2	29 0 ± 1 8	30 6 ± 1 6
200 + 0	30 0 ± 1 2	29 8 ± 1 6	28 6 ± 1 6	30 9 ± 1 7
400 + 0	29 4 ± 1 4	30 0 ± 1 3	29 2 ± 1 7	30 8 ± 1 6
0 + 250	29 7 ± 1 7	29 6 ± 1 6	29 8 ± 1 4	30 6 ± 1 4
0 + 500	29 8 ± 1 9	29 6 ± 1 5	30 2 ± 1 6	31 5 ± 1 7
0 + 1,000	30 8 ± 1 8	30 4 ± 2 2	30 0 ± 1 9	30 4 ± 2 8
200 + 250	30 2 ± 1 8	30 4 ± 1 7	30 3 ± 1 7	31 2 ± 1 6
200 + 500	30 4 ± 1 5	30 0 ± 1 8	30 6 ± 1 9	30 8 ± 1 6
200 + 1,000	30 4 ± 1 4	30 6 ± 1 5	30 2 ± 1 5	29 9 ± 2 3
400 + 250	30 0 ± 1 7	30 0 ± 1 6	29 9 ± 1 9	30 3 ± 1 6
400 + 500	30 5 ± 1 7	30 3 ± 1 9	30 0 ± 1 4 (19)	30 5 ± 1 7 (19)
400 + 1,000	30 4 ± 1 4	30 8 ± 1 6	29 4 ± 1 5	29 2 ± 1 5

	Mean Body Weights[b] on Study Day		
Dose[a]	**20**	**23**	**26**
0 + 0	35 1 ± 2 3	40 2 ± 2 9	48 0 ± 4 3
200 + 0	33 2 ± 3 1	36 4 ± 5 0	41 0 ± 7 8*
400 + 0	32 5 ± 2 4	34 6 ± 3 2**	37 2 ± 4 9**
0 + 250	34 4 ± 2 5	38 8 ± 4 2	46 2 ± 6 2
0 + 500	35 2 ± 3 0	39 3 ± 4 7	46 0 ± 7 4
0 + 1,000	34 5 ± 2 7 (19)	37 2 ± 5 1 (19)	42 9 ± 8 3 (19)
200 + 250	34 4 ± 3 0	37 8 ± 4 4	43 0 ± 6 8
200 + 500	33 2 ± 2 5	35 4 ± 3 6**	39 0 ± 5 4**
200 + 1,000	31 8 ± 3 2	33 4 ± 5 0**	35 8 ± 6 8**
400 + 250	32 8 ± 2 4	33 9 ± 3 8**	35 8 ± 5 7**
400 + 500	31 6 ± 2 3 (19)	30 9 ± 2 7**▲ (19)	31 2 ± 3 6**,▲▲ (19)
400 + 1,000	29 4 ± 2 4**	29 6 ± 2 2**▲▲ (19)	29 7 ± 3 0**,▲▲ (19)

[a] AZT + clarithromycin in mg/kg per day
[b] Weight (grams) expressed as group mean ± standard deviation, n = 20 unless otherwise noted in parentheses
* P≤0 05 compared to 0 + 0 (control) group by Scheffe's or Dunn's test
** P≤0 01 compared to 0 + 0 (control) group by Scheffe's or Dunn's test
▲ P≤0 05 compared to 400 + 0 group by Scheffe's or Dunn's test
▲▲ P≤0 01 compared to 400 + 0 group by Scheffe's or Dunn's test

TABLE A1
Summary of Body Weights for Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations

Female-B Mice

		Mean Body Weights[c] on Day of Gestation			
Dose[a]	n[b]	0	8	12	15
0 + 0	15	30 7 ± 1 8[d]	35 0 ± 1 9	40 2 ± 4 1	48 3 ± 3 4 (14)
200 + 0	14	30 0 ± 1 7	34 8 ± 2 1	40 1 ± 3 3	47 6 ± 5 3
400 + 0	16	28 6 ± 1 7	33 2 ± 2 1	39 4 ± 1 7	46 8 ± 2 2
0 + 250	15	28 5 ± 1 4	33 1 ± 1 8	39 5 ± 2 2	46 7 ± 3 3
0 + 500	12	28 8 ± 1 4	34 2 ± 2 3 (11)	40 9 ± 3 1 (11)	49 3 ± 3 5 (11)
0 + 1,000	14	29 2 ± 1 7[d]	34 2 ± 1 9	39 8 ± 2 6	47 3 ± 3 1
200 + 250	15	29 7 ± 1 2	34 4 ± 1 5	40 5 ± 2 1	47 6 ± 3 4
200 + 500	14	29 6 ± 1 1	33 8 ± 1 2	39 4 ± 2 4	45 2 ± 3 7
200 + 1,000	11	29 4 ± 1 7	34 4 ± 1 6	38 4 ± 1 8	44 1 ± 2 7
400 + 250	14	28 8 ± 1 5	34 6 ± 1 6	40 3 ± 2 7	44 1 ± 4 3
400 + 500	15	29 5 ± 1 5	34 9 ± 2 1	40 3 ± 3 7	44 7 ± 4 7
400 + 1,000	15	29 7 ± 1 9	34 7 ± 2 0	38 8 ± 2 6	42 1 ± 3 9*

		Mean Body Weights[c] on Day of Lactation		
Dose[a]	n[b]	0	1	4
0 + 0	15	36 6 ± 2 3 (14)	36 7 ± 2 2 (14)	42 9 ± 3 7 (14)
200 + 0	14	35 8 ± 2 3	36 1 ± 3 6	40 7 ± 3 5
400 + 0	16	35 3 ± 1 2	35 4 ± 1 6	40 8 ± 2 7
0 + 250	15	35 1 ± 2 0	35 5 ± 2 0	41 7 ± 2 9
0 + 500	12	35 7 ± 1 8 (11)	37 1 ± 2 0 (11)	41 5 ± 3 2 (11)
0 + 1,000	14	35 7 ± 2 2	36 1 ± 2 4 (13)	40 4 ± 3 5 (12)
200 + 250	15	35 9 ± 2 3	35 8 ± 2 6	40 2 ± 3 5 (14)
200 + 500	14	35 7 ± 1 7 (7)	35 1 ± 2 5 (7)	39 2 ± 2 6 (6)
200 + 1,000	11	33 2 ± 2 4 (8)	32 8 ± 3 2 (5)	34 4 ± 4 4** (5)
400 + 250	14	35 7 ± 3 6 (6)	34 8 ± 3 8 (5)	35 0 ± 0 0 (2)
400 + 500	15	35 2 ± 1 0 (4)	34 2 ± 1 0 (4)	35 0 ± 5 6* (4)
400 + 1,000	15	(0)	(0)	(0)

[a] AZT + clarithromycin in mg/kg per day
[b] n = number of pregnant females in group unless otherwise noted in parentheses, dams with no surviving pups were excluded from lactation-period weights
[c] Weight (grams) expressed as group mean ± standard deviation
[d] One value not recorded
* $P \leq 0.05$ compared to 0 + 0 (control) group by Scheffe's or Dunn's test
** $P \leq 0.01$ compared to 0 + 0 (control) group by Scheffe's or Dunn's test

TABLE A2
Summary of Organ Weights for Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations

Male Mice

Dose[a]	n[b]	Body Weight[c]	Liver Weight[d] Absolute	Liver Weight[d] Relative
0 + 0	10	37 31	1 8870 ± 0 1718 (NA)	50 7 ± 5 4 (NA)
200 + 0	10	38 31	1 9110 ± 0 2618 (+1 3)	49 8 ± 6 0 (-1 7)
400 + 0	10	38 33	1 9690 ± 0 2277 (+4 3)	51 4 ± 5 2 (+1 3)
0 + 250	10	38 45	1 8560 ± 0 1841 (-1 6)	48 3 ± 4 1 (-4 8)
0 + 500	10	38 40	1 8290 ± 0 1826 (-3 1)	47 6 ± 3 5 (-6 2)
0 + 1,000	10	39 02	2 0970 ± 0 2450 (+11 1)*	53 7 ± 4 8 (+5 9)
200 + 250	10	37 98	1 8130 ± 0 2097 (-3 9)	47 8 ± 5 4 (-5 8)
200 + 500	10	38 69	1 9250 ± 0 2290 (+2 0)	49 7 ± 4 9 (-2 0)
200 + 1,000	10	38 22	1 9470 ± 0 1543 (+3 2)	50 9 ± 3 7 (+0 5)
400 + 250	10	39 15*	1 9210 ± 0 1926 (+1 8)	49 0 ± 4 0 (-3 3)
400 + 500	10	38 77	1 9240 ± 0 2408 (+2 0)	49 6 ± 5 6 (-2 1)
400 + 1,000	10	38 10	1 9940 ± 0 2212 (+5 7)	52 5 ± 5 5 (+3 5)

Dose[a]	n[b]	Reproductive Organ Weight[e] Left Testis	Reproductive Organ Weight[e] Left Epididymis
0 + 0	10	0 1256 ± 0 0042[f]	0 0476 ± 0 0026
200 + 0	10	0 1272 ± 0 0062	0 0512 ± 0 0013
400 + 0	10	0 1148 ± 0 0065	0 0486 ± 0 0015
0 + 250	10	0 1270 ± 0 0037	0 0498 ± 0 0015
0 + 500	10	0 1173 ± 0 0040	0 0490 ± 0 0013
0 + 1,000	10	0 1227 ± 0 0060	0 0500 ± 0 0012
200 + 250	10	0 1181 ± 0 0071	0 0491 ± 0 0023
200 + 500	10	0 1266 ± 0 0059	0 0499 ± 0 0024
200 + 1,000	10	0 1221 ± 0 0070	0 0485 ± 0 0019
400 + 250	10	0 1205 ± 0 0028	0 0504 ± 0 0016
400 + 500	10	0 1225 ± 0 0052	0 0524 ± 0 0015
400 + 1,000	10	0 1190 ± 0 0065	0 0470 ± 0 0016

[a] AZT + clarithromycin in mg/kg per day
[b] n = number of values included in calculations, unless otherwise indicated
[c] Group mean body weight in grams
[d] Liver weights are presented as group mean ± standard deviation (% difference from control group, NA = not applicable) Absolute weights in grams, relative weights (organ-weight-to-body-weight ratios) are mg liver weight/g body weight
[e] Reproductive organ weights are presented in grams as group mean ± standard error
[f] n = 9
* P≤0 05 compared to 0 + 0 (control) group by the Student's *t*-test

TABLE A2
Summary of Organ Weights for Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations

Female-A Mice

		Body	Liver Weight[d]	
Dose[a]	n[b]	Weight[c]	Absolute	Relative
0 + 0	18	36 87	2 457 ± 0 247 (NA)	66 6 ± 5 3 (NA)
200 + 0	16	37 79	2 484 ± 0 299 (+1 1)	65 6 ± 6 1 (-1 5)
400 + 0	15	36 43	2 223 ± 0 419 (-9 5)*	60 6 ± 7 2 (-9 0)*
0 + 250	16	37 46	2 592 ± 0 170 (+5 5)	69 2 ± 4 0 (+3 9)
0 + 500	15	37 35	2 526 ± 0 199 (+2 8)	67 6 ± 2 8 (+1 5)
0 + 1,000	12	37 26	2 593 ± 0 324 (+5 5)	69 5 ± 6 3 (+4 4)
200 + 250	18	37 89	2 441 ± 0 329 (-0 7)	64 3 ± 6 7 (-3 4)
200 + 500	16	38 51	2 464 ± 0 282 (+0 3)	63 9 ± 5 3 (-4 1)
200 + 1,000	9	37 57	2 420 ± 0 439 (-1 5)	64 1 ± 8 8 (-3 8)
400 + 250	13	33 75*	2 173 ± 0 547 (-11 6)*	65 7 ± 18 1 (-1 4)
400 + 500	4	34 75	1 753 ± 0 356 (-28 7)*	50 3 ± 2 8 (-24 5)*
400 + 1,000	2	35 65	2 385 ± 0 191 (-2 9)	66 9 ± 5 2 (+0 4)

[a] AZT + clarithromycin in mg/kg per day
[b] n = number of values included in calculations Values exclude early deaths, dams designated as not pregnant, dams that delivered or began delivery prior to scheduled sacrifice, and dams that were sacrificed on an estimated day 14 or 18 of gestation
[c] Group mean body weight in grams
[d] Liver weights are presented as group mean ± standard deviation (% difference from control group, NA = not applicable) Absolute weights in grams, relative weights (organ-weight-to-body-weight ratios) are mg liver weight/g body weight
* $P \leq 0.05$ compared to 0 + 0 (control) group by the Student's *t*-test

APPENDIX B
CLINICAL PATHOLOGY

List of Abbreviations

WBC	White blood cells
RBC	Red blood cells
Hgb	Hemoglobin
Hct	Hematocrit
MCV	Mean corpuscular volume
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
Plt	Platelets
Retic	Reticulocytes
Neut	Neutrophils
Lymph	Lymphocytes
Mono	Monocytes
Eos	Eosinophils
Baso	Basophils
LUC	Large unstained cells
RDW	Red cell distribution width
BUN	Blood urea nitrogen
Crea	Creatinine
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BilA	Total bile acids

TABLE B1
Summary of Hematology Parameters for Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations

Male Mice

	WBC $10^3/\mu L$	RBC $10^6/\mu L$	Hgb g/dL	Hct %	MCV fL	MCH pg	MCHC g/dL	Plt $10^3/\mu L$
0 + 0 mg/kg per day[a]								
Mean	6 10	10 34	16 4	47 9	46 5	15 9	34 2	838
SD	1 469	0 795	1 14	3 22	2 41	0 72	0 64	253 3
n	10	10	10	10	10	10	10	10
200 + 0 mg/kg per day								
Mean	5 74	9 29	15 4	46 6	50 3*	16 7	33 2	920
SD	1 642	0 936	1 25	3 85	2 64	0 70	0 90	158 9
n	10	10	10	10	10	10	10	10
400 + 0 mg/kg per day								
Mean	4 87	7 95**	13 8**	41 6*	52 4**	17 5**	33 3	1,131
SD	1 072	1 009	1 47	4 52	1 90	0 54	0 58	230 2
n	10	10	10	10	10	10	10	10
0 + 250 mg/kg per day								
Mean	6 91	9 84	16 0	47 1	47 9	16 2	33 9	922
SD	2 109	0 503	0 97	2 84	1 86	0 58	0 74	107 7
n	10	10	10	10	10	10	10	10
0 + 500 mg/kg per day								
Mean	7 29	9 71	15 7	45 6	47 3	16 2	34 5	983
SD	1 549	0 956	0 81	2 70	3 51	1 07	0 81	202 1
n	10	10	10	10	10	10	10	10
0 + 1,000 mg/kg per day								
Mean	7 14	9 80	15 9	46 3	47 3	16 2	34 3	1,034
SD	2 872	0 623	1 04	2 75	1 36	0 26	1 07	345 4
n	10	10	10	10	10	10	10	10
200 + 250 mg/kg per day								
Mean	5 07	8 30**	14 3*	42 2	51 0**	17 3**	33 8	1,236
SD	2 274	0 794	1 17	3 79	2 61	0 88	0 68	282 7
n	10	10	10	10	10	10	10	10
200 + 500 mg/kg per day								
Mean	5 02	7 82**	13 3**	39 6**	50 6**	17 1**	33 7	1,095
SD	1 679	1 097	1 93	6 07	2 65	0 59	0 81	280 7
n	10	10	10	10	10	10	10	10
200 + 1,000 mg/kg per day								
Mean	5 29	7 56**	12 7**	37 2**	48 9	16 7	34 2	1,141
SD	1 764	0 947	1 93	6 67	3 65	0 71	1 45	165 3
n	10	10	10	10	10	10	10	10

TABLE B1
Summary of Hematology Parameters for Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations

Male Mice

	WBC $10^3/\mu L$	RBC $10^6/\mu L$	Hgb g/dL	Hct %	MCV fL	MCH pg	MCHC g/dL	Plt $10^3/\mu L$
400 + 250 mg/kg per day								
Mean	4 31	6 94**	11 8**	35 1**	50 6*	17 0**	33 7	1,299
SD	1 368	1 081	1 88	6 15	2 16	0 67	0 96	306 7
n	10	10	10	10	10	10	10	10
400 + 500 mg/kg per day								
Mean	5 75	7 02**	11 9**	35 0**	49 7	16 9*	34 0	1,412
SD	1 747	1 244	2 24	7 06	2 81	0 64	0 82	549 4
n	10	10	10	10	10	10	10	10
400 + 1,000 mg/kg per day								
Mean	5 58	5 95**	9 9**	28 5**	47 5	16 5	34 9	1,343
SD	3 336	1 245	2 39	7 83	4 29	0 93	1 40	578 5
n	10	10	10	10	10	10	10	10

	Retic $10^5/\mu L$	Neut $10^3/\mu L$	Lymph $10^3/\mu L$	Mono $10^3/\mu L$	Eos $10^3/\mu L$	Baso $10^3/\mu L$	LUC $10^3/\mu L$	RDW %
0 + 0 mg/kg per day								
Mean	4 0	1 00	4 76	0 09	0 16	0 03	0 07	13 9
SD	0 64	0 582	1 011	0 031	0 090	0 018	0 073	0 80
n	10	10	10	10	10	10	10	10
200 + 0 mg/kg per day								
Mean	4 1	0 75	4 66	0 08	0 17	0 02	0 06	19 8**
SD	0 75	0 439	1 529	0 032	0 097	0 012	0 038	2 38
n	10	10	10	10	10	10	10	10
400 + 0 mg/kg per day								
Mean	4 0	0 60	3 98	0 10	0 13	0 01	0 07	22 3**
SD	1 66	0 289	0 894	0 055	0 081	0 008	0 039	2 29
n	9	10	10	10	10	10	8	10
0 + 250 mg/kg per day								
Mean	4 7	0 89	5 71	0 09	0 14	0 03	0 06	14 3
SD	2 34	0 338	1 782	0 036	0 045	0 011	0 029	0 78
n	10	10	10	10	10	10	10	10
0 + 500 mg/kg per day								
Mean	4 1	1 18	5 69	0 11	0 21	0 03	0 09	14 3
SD	0 70	0 425	1 425	0 051	0 138	0 016	0 043	0 49
n	9	10	10	10	10	10	9	10

TABLE B1
Summary of Hematology Parameters for Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations

Male Mice

	Retic $10^5/\mu L$	Neut $10^3/\mu L$	Lymph $10^3/\mu L$	Mono $10^3/\mu L$	Eos $10^3/\mu L$	Baso $10^3/\mu L$	LUC $10^3/\mu L$	RDW %
0 + 1,000 mg/kg per day								
Mean	4 3	1 07	5 49	0 12	0 27	0 03	0 16	14 5
SD	0 62	0 522	2 313	0 057	0 171	0 018	0 200	0 43
n	9	10	10	10	10	10	10	10
200 + 250 mg/kg per day								
Mean	3 4	0 68	4 04	0 08	0 20	0 02	0 06	21 7**
SD	1 09	0 677	1 760	0 039	0 108	0 009	0 038	1 82
n	9	10	10	10	10	10	10	10
200 + 500 mg/kg per day								
Mean	3 2	0 77	3 93	0 09	0 17	0 01	0 05	23 1**
SD	1 16	0 773	1 351	0 071	0 094	0 007	0 025	2 69
n	9	10	10	10	10	10	9	10
200 + 1,000 mg/kg per day								
Mean	3 9	0 59	4 30	0 08	0 20	0 02	0 11	21 5**
SD	1 76	0 259	1 460	0 045	0 164	0 008	0 085	4 40
n	8	10	10	10	10	10	9	10
400 + 250 mg/kg per day								
Mean	2 6	0 41	3 65	0 06	0 12	0 01	0 06	24 2**
SD	1 22	0 211	1 179	0 027	0 044	0 005	0 033	2 11
n	10	10	10	10	10	10	10	10
400 + 500 mg/kg per day								
Mean	4 1	0 76	4 71	0 08	0 12	0 01	0 08	24 2**
SD	2 86	0 658	1 712	0 039	0 076	0 011	0 047	4 22
n	9	10	10	10	10	10	7	10
400 + 1,000 mg/kg per day								
Mean	2 4	1 17	4 01	0 12	0 19	0 02	0 10	21 7**
SD	1 97	1 580	2 595	0 131	0 144	0 015	0 076	6 96
n	9	10	10	10	10	10	8	10

[a] AZT + clarithromycin

* Significantly different (P<0 05) from the control group by Dunnett's test

** Significantly different (P<0 01) from the control group by Dunnett's test

TABLE B1
Summary of Hematology Parameters for Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations

Female-A Mice

	WBC $10^3/\mu L$	RBC $10^6/\mu L$	Hgb g/dL	Hct %	MCV fL	MCH pg	MCHC g/dL	Plt $10^3/\mu L$
0 + 0 mg/kg per day[a]								
Mean	5 92	8 57	14 4	42 0	49 0	16 8	34 4	1,034
SD	1 226	0 553	1 43	3 54	2 78	1 04	1 26	191 1
n	19	19	19	19	19	19	19	19
200 + 0 mg/kg per day								
Mean	5 23	7 60	14 3	42 1	55 5**	18 8**	34 0	1,126
SD	1 561	0 664	1 08	3 38	2 92	0 98	1 27	226 1
n	20	20	20	20	20	20	20	20
400 + 0 mg/kg per day								
Mean	6 75	7 75	14 2	42 6	55 3**	18 4**	33 3	1,065
SD	1 431	1 018	1 55	4 46	3 62	0 84	1 49	195 7
n	12	12	12	12	12	12	12	12
0 + 250 mg/kg per day								
Mean	7 47	8 78	14 8	43 5	49 6	16 8	33 9	1,134
SD	1 946	0 527	1 47	3 03	1 51	0 83	1 50	248 0
n	11	11	11	11	11	11	11	11
0 + 500 mg/kg per day								
Mean	7 99	8 89	14 5	42 5	47 8	16 3	34 2	1,076
SD	3 106	1 187	2 17	5 82	1 07	0 52	1 13	212 2
n	12	12	12	12	12	12	12	12
0 + 1,000 mg/kg per day								
Mean	12 74**	9 21	14 8	43 9	47 7	16 1	33 8	821
SD	7 679	0 784	1 70	4 24	2 65	0 79	1 95	275 6
n	11	11	11	11	11	11	11	11
200 + 250 mg/kg per day								
Mean	5 61	6 59**	12 5	38 0	57 6**	19 1**	33 2	1,442**
SD	1 022	0 844	1 64	5 65	5 44	1 29	1 89	335 2
n	20	20	20	20	20	20	20	20
200 + 500 mg/kg per day								
Mean	6 23	6 66**	13 0	39 6	59 7**	19 6**	32 8	1,332
SD	2 350	0 857	1 48	3 94	4 14	1 13	1 15	342 9
n	20	20	20	20	20	20	20	20
200 + 1,000 mg/kg per day								
Mean	6 47	6 01**	11 0**	32 5**	53 0	18 0**	34 3	1,386*
SD	3 000	1 244	2 83	9 85	6 92	1 35	2 54	338 6
n	20	20	20	20	20	20	20	20

TABLE B1
Summary of Hematology Parameters for Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations

Female-A Mice

	WBC $10^3/\mu L$	RBC $10^6/\mu L$	Hgb g/dL	Hct %	MCV fL	MCH pg	MCHC g/dL	Plt $10^3/\mu L$
400 + 250 mg/kg per day								
Mean	5 59	5 22**	9 7**	29 5**	53 6*	18 0*	34 0	1,758**
SD	1 505	2 183	4 46	14 82	8 73	1 69	3 14	653 2
n	12	12	12	12	12	12	12	12
400 + 500 mg/kg per day								
Mean	4 36	2 45**	4 0**	11 0**	44 5	16 4	36 9**	1,982**
SD	1 158	0 692	1 22	3 43	1 43	0 95	2 16	808 8
n	12	12	12	12	12	12	12	12
400 + 1,000 mg/kg per day								
Mean	3 87	3 66**	6 3**	17 6**	46 8	17 0	36 6*	1,645**
SD	1 655	2 196	3 93	11 85	5 77	1 51	1 78	455 6
n	10	10	10	10	10	10	10	10

	Retic $10^5/\mu L$	Neut $10^3/\mu L$	Lymph $10^3/\mu L$	Mono $10^3/\mu L$	Eos $10^3/\mu L$	Baso $10^3/\mu L$	LUC $10^3/\mu L$	RDW %
0 + 0 mg/kg per day								
Mean	3 9	1 71	3 92	0 09	0 10	0 02	0 07	16 0
SD	1 16	0 589	1 033	0 028	0 036	0 008	0 030	1 19
n	19	19	19	19	19	19	19	19
200 + 0 mg/kg per day								
Mean	3 9	1 16	3 76	0 09	0 14	0 01	0 07	19 9
SD	1 34	0 477	1 171	0 048	0 141	0 009	0 041	1 63
n	19	20	20	20	20	20	18	20
400 + 0 mg/kg per day								
Mean	4 0	1 13	5 21*	0 12	0 18	0 02	0 09	21 1*
SD	1 23	0 307	1 461	0 035	0 065	0 010	0 027	1 75
n	12	12	12	12	12	12	12	12
0 + 250 mg/kg per day								
Mean	3 9	2 33	4 72	0 12	0 15	0 02	0 13	15 8
SD	1 02	0 907	1 323	0 032	0 048	0 010	0 072	1 18
n	9	11	11	11	11	11	11	11
0 + 500 mg/kg per day								
Mean	3 4	2 57	4 97	0 13	0 18	0 03	0 12	15 4
SD	1 27	2 055	1 200	0 086	0 116	0 009	0 086	0 67
n	11	12	12	12	12	12	12	12

TABLE B1
Summary of Hematology Parameters for Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations

Female-A Mice

	Retic $10^5/\mu L$	Neut $10^3/\mu L$	Lymph $10^3/\mu L$	Mono $10^3/\mu L$	Eos $10^3/\mu L$	Baso $10^3/\mu L$	LUC $10^3/\mu L$	RDW %
0 + 1,000 mg/kg per day								
Mean	3 3	5 71**	5 89**	0 22**	0 46**	0 06**	0 40**	15 4
SD	1 54	6 298	1 768	0 124	0 306	0 062	0 530	0 84
n	11	11	11	11	11	11	11	11
200 + 250 mg/kg per day								
Mean	4 4	1 36	3 92	0 09	0 13	0 01	0 11	25 8**
SD	2 59	0 488	0 758	0 031	0 066	0 006	0 050	5 36
n	20	20	20	20	20	20	19	20
200 + 500 mg/kg per day								
Mean	4 1	1 70	4 10	0 11	0 20	0 02	0 10	26 7**
SD	1 41	1 577	1 089	0 081	0 149	0 011	0 045	5 03
n	19	20	20	20	20	20	19	20
200 + 1,000 mg/kg per day								
Mean	4 0	1 75	4 18	0 10	0 23*	0 02	0 20	24 6**
SD	2 48	2 210	1 049	0 084	0 157	0 013	0 172	7 00
n	19	20	20	20	20	20	19	20
400 + 250 mg/kg per day								
Mean	3 5	0 98	4 27	0 09	0 14	0 01	0 11	25 4**
SD	3 01	0 651	1 316	0 061	0 089	0 008	0 051	5 74
n	12	12	12	12	12	12	11	12
400 + 500 mg/kg per day								
Mean	0 6**	0 23	3 97	0 02*	0 04	0 01	0 08	16 1
SD	0 52	0 231	1 037	0 016	0 053	0 006	0 056	5 66
n	11	12	12	12	12	12	11	12
400 + 1,000 mg/kg per day								
Mean	1 9	0 54	3 09	0 05	0 11	0 01	0 09	19 3
SD	2 91	0 784	0 888	0 041	0 158	0 006	0 080	9 14
n	10	10	10	10	10	10	8	10

[a] AZT + clarithromycin

* Significantly different (P<0 05) from the control by Dunnett's test

** Significantly different (P<0 01) from the control by Dunnett's test

TABLE B1
Summary of Hematology Parameters for Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations

Female-B Mice

	WBC $10^3/\mu L$	RBC $10^6/\mu L$	Hgb g/dL	Hct %	MCV fL	MCH pg	MCHC g/dL	Plt $10^3/\mu L$
0 + 0 mg/kg per day[a]								
Mean	4 92	8 26	14 2	42 5	51 6	17 2	33 3	1,100
SD	1 615	0 658	1 11	2 57	2 28	0 66	0 88	253 2
n	14	14	14	14	14	14	14	14
200 + 0 mg/kg per day								
Mean	5 17	7 94	14 5	43 0	54 4**	18 3**	33 7	1,093
SD	1 277	0 840	1 34	3 07	2 35	0 65	1 44	328 9
n	15	15	15	15	15	15	15	15
400 + 0 mg/kg per day								
Mean	4 72	7 69	14 1	42 0	54 7**	18 3**	33 5	1,089
SD	1 123	0 367	0 52	1 42	1 83	0 60	0 70	196 0
n	16	16	16	16	16	16	16	16
0 + 250 mg/kg per day								
Mean	5 40	8 30	14 5	43 3	52 3	17 5	33 5	1,146
SD	1 376	0 488	0 71	2 48	2 55	0 60	1 27	308 7
n	15	15	15	15	15	15	15	15
0 + 500 mg/kg per day								
Mean	5 00	8 88	15 4	45 1	50 9	17 3	34 0	1,121
SD	1 435	0 645	1 51	3 54	2 26	0 76	1 58	287 2
n	13	13	13	13	13	13	13	13
0 + 1,000 mg/kg per day								
Mean	5 24	8 80	15 4	45 6	51 8	17 5	33 8	1,196
SD	1 492	0 482	0 92	2 64	1 67	0 59	0 90	364 0
n	15	15	15	15	15	15	15	15
200 + 250 mg/kg per day								
Mean	5 53	7 92	14 6	43 7	55 3**	18 5**	33 4	1,303
SD	2 071	0 563	1 02	3 12	2 48	0 72	1 31	166 6
n	15	15	15	15	15	15	15	15
200 + 500 mg/kg per day								
Mean	5 47	8 16	15 2	45 2	55 4**	18 7**	33 7	1,193
SD	1 693	0 809	1 66	4 37	2 19	0 78	1 27	295 7
n	14	14	14	14	14	14	14	14
200 + 1,000 mg/kg per day								
Mean	5 30	8 41	15 4	45 9	54 8**	18 4**	33 6	1,066
SD	1 278	0 892	1 37	4 16	2 72	0 77	0 87	335 7
n	14	14	14	14	14	14	14	14

TABLE B1
Summary of Hematology Parameters for Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations

Female-B Mice

	WBC $10^3/\mu L$	RBC $10^6/\mu L$	Hgb g/dL	Hct %	MCV fL	MCH pg	MCHC g/dL	Plt $10^3/\mu L$
400 + 250 mg/kg per day								
Mean	6 03	8 14	15 2	43 7	53 7	18 6**	34 7	1,185
SD	1 384	0 731	1 36	4 07	2 45	0 81	0 67	286 6
n	12	12	12	12	12	12	12	12
400 + 500 mg/kg per day								
Mean	5 01	8 73	16 2	47 3	54 3*	18 5*	34 1	1,149
SD	1 555	0 790	1 29	3 78	2 01	0 72	0 77	339 2
n	15	15	15	15	15	15	15	15
400 + 1,000 mg/kg per day								
Mean	5 96	8 12	15 0	44 2	54 5**	18 5**	33 9	1,105
SD	2 008	1 214	2 25	6 58	2 52	0 50	0 84	386 0
n	15	15	15	15	15	15	15	15

	Retic $10^5/\mu L$	Neut $10^3/\mu L$	Lymph $10^3/\mu L$	Mono $10^3/\mu L$	Eos $10^3/\mu L$	Baso $10^3/\mu L$	LUC $10^3/\mu L$	RDW %
0 + 0 mg/kg per day								
Mean	4 4	1 06	3 55	0 08	0 16	0 01	0 07	17 1
SD	1 43	0 619	0 949	0 042	0 128	0 008	0 053	1 49
n	13	14	14	14	14	14	12	14
200 + 0 mg/kg per day								
Mean	6 8	1 22	3 62	0 08	0 17	0 02	0 07	19 9**
SD	2 34	0 489	1 045	0 023	0 118	0 009	0 038	1 81
n	14	15	15	15	15	15	15	15
400 + 0 mg/kg per day								
Mean	6 0	0 99	3 45	0 08	0 13	0 01	0 06	21 4**
SD	1 57	0 380	0 934	0 027	0 072	0 008	0 031	1 61
n	16	16	16	16	16	16	15	16
0 + 250 mg/kg per day								
Mean	4 7	1 12	4 00	0 08	0 12	0 01	0 09	17 4
SD	1 35	0 589	1 098	0 026	0 051	0 007	0 059	1 04
n	15	15	15	15	15	15	13	15
0 + 500 mg/kg per day								
Mean	4 1	0 92	3 81	0 08	0 12	0 01	0 07	16 3
SD	1 35	0 401	1 073	0 038	0 056	0 007	0 048	1 15
n	12	13	13	13	13	13	12	13

TABLE B1
Summary of Hematology Parameters for Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations

Female-B Mice

	Retic $10^5/\mu L$	Neut $10^3/\mu L$	Lymph $10^3/\mu L$	Mono $10^3/\mu L$	Eos $10^3/\mu L$	Baso $10^3/\mu L$	LUC $10^3/\mu L$	RDW %
0 + 1,000 mg/kg per day								
Mean	3 8	1 10	3 84	0 08	0 14	0 01	0 07	16 7
SD	1 29	0 651	0 905	0 052	0 131	0 011	0 055	1 23
n	15	15	15	15	15	15	14	15
200 + 250 mg/kg per day								
Mean	6 2	1 22	4 02	0 07	0 12	0 02	0 07	22 0**
SD	2 24	0 977	1 482	0 054	0 042	0 009	0 052	1 03
n	15	15	15	15	15	15	15	15
200 + 500 mg/kg per day								
Mean	4 7	0 99	4 10	0 08	0 21	0 02	0 08	22 8**
SD	3 47	0 403	1 296	0 049	0 150	0 009	0 039	2 50
n	14	14	14	14	14	14	13	14
200 + 1,000 mg/kg per day								
Mean	4 4	1 02	3 93	0 09	0 19	0 02	0 06	21 9**
SD	2 69	0 555	0 978	0 043	0 106	0 011	0 036	2 69
n	14	14	14	14	14	14	13	14
400 + 250 mg/kg per day								
Mean	5 4	1 17	4 44	0 09	0 20	0 02	0 10	22 3**
SD	4 78	0 283	1 256	0 046	0 078	0 011	0 050	1 83
n	12	12	12	12	12	12	12	12
400 + 500 mg/kg per day								
Mean	5 2	1 02	3 69	0 08	0 14	0 02	0 07	22 2**
SD	2 24	0 665	1 168	0 065	0 065	0 008	0 046	2 09
n	15	15	15	15	15	15	14	15
400 + 1,000 mg/kg per day								
Mean	5 0	1 02	4 53	0 09	0 17	0 03	0 14	23 9**
SD	2 64	0 251	1 721	0 029	0 101	0 025	0 158	2 60
n	15	15	15	15	15	15	13	15

[a] AZT + clarithromycin

* Significantly different (P<0 05) from the control by Dunnett's test

** Significantly different (P<0 01) from the control by Dunnett's test

TABLE B2

Summary of Clinical Chemistry Parameters for Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations

Male Mice

	BUN mg/dL	Crea mg/dL	ALP U/L	ALT U/L	AST U/L	BilA µmol/L
0 + 0 mg/kg per day[a]						
Mean	24 2	0 40	60	32	NA	12
SD	5 44	0 106	16 8	10 8	NA	10 3
n	10	10	9	9	0	5
200 + 0 mg/kg per day						
Mean	23 8	0 41	62	31	82	23
SD	4 31	0 113	9 9	6 3	12 7	13 5
n	10	10	9	7	2	5
400 + 0 mg/kg per day						
Mean	26 6	0 46	44	31	95	15
SD	4 07	0 214	11 6	8 1	39 7	13 8
n	10	10	9	8	3	7
0 + 250 mg/kg per day						
Mean	23 6	0 39	54	35	104	26
SD	3 05	0 077	15 0	11 0	6 0	13 8
n	10	8	9	7	3	6
0 + 500 mg/kg per day						
Mean	24 1	0 42	53	35	NA	16
SD	2 82	0 106	15 5	8 1	NA	13 5
n	9	9	8	9	0	7
0 + 1,000 mg/kg per day						
Mean	23 1	0 44	47	58	62	20
SD	6 32	0 185	10 8	33 2	4 9	25 6
n	10	9	9	8	2	4
200 + 250 mg/kg per day						
Mean	24 2	0 38	45	29	63	24
SD	5 56	0 099	10 5	5 4	2 8	17 9
n	10	10	10	8	2	5
200 + 500 mg/kg per day						
Mean	21 1	0 39	53	35	112	26
SD	3 40	0 083	19 4	11 5	62 0	12 2
n	10	10	7	10	4	7
200 + 1,000 mg/kg per day						
Mean	21 1	0 36	51	82	109	25
SD	3 92	0 101	20 7	84 2	46 1	16 3
n	10	9	9	7	4	5

TABLE B2

Summary of Clinical Chemistry Parameters for Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations

Male Mice

	BUN mg/dL	Crea mg/dL	ALP U/L	ALT U/L	AST U/L	BilA µmol/L
400 + 250 mg/kg per day						
Mean	31 8	0 47	59	28	65	16
SD	10 81	0 203	20 0	8 7	9 3	14 4
n	10	10	10	9	5	8
400 + 500 mg/kg per day						
Mean	21 0	0 39	52	41	74	13
SD	3 15	0 094	11 5	25 3	15 6	6 7
n	9	9	7	6	2	6
400 + 1,000 mg/kg per day						
Mean	24 7	0 44	55	44	129	18
SD	4 31	0 077	23 2	13 1	89 1	14 3
n	10	10	10	9	3	8

[a] AZT + clarithromycin

* Significantly different (P<0 05) from the control by Dunnett's test

** Significantly different (P<0 01) from the control by Dunnett's test

NA Not applicable

TABLE B2
Summary of Clinical Chemistry Parameters for Swiss (CD-1) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations

Female-A Mice

	BUN mg/dL	Crea mg/dL	ALP U/L	ALT U/L	AST U/L	BilA µmol/L
0 + 0 mg/kg per day[a]						
Mean	18 3	0 39	52	36	102	11
SD	3 97	0 094	16 5	9 1	35 9	7 6
n	19	19	18	19	12	16
200 + 0 mg/kg per day						
Mean	20 4	0 38	59	31	80	11
SD	2 92	0 057	13 4	9 1	15 5	6 0
n	20	20	20	19	11	14
400 + 0 mg/kg per day						
Mean	21 6	0 40	70	25	72	11
SD	2 11	0 054	26 2	5 3	16 0	6 8
n	12	11	12	11	7	11
0 + 250 mg/kg per day						
Mean	19 8	0 39	46	35	77	9
SD	2 82	0 055	11 0	12 1	36 2	4 6
n	11	11	11	10	5	7
0 + 500 mg/kg per day						
Mean	19 5	0 39	42	32	85	9
SD	2 93	0 055	10 4	8 5	10 5	6 7
n	12	12	11	11	6	8
0 + 1,000 mg/kg per day						
Mean	23 5**	0 45	53	52*	102	11
SD	5 65	0 085	21 5	20 9	32 2	5 9
n	10	9	10	8	4	6
200 + 250 mg/kg per day						
Mean	20 5	0 39	59	30	105	17
SD	3 51	0 083	19 9	10 2	25 6	10 8
n	20	20	20	20	17	18
200 + 500 mg/kg per day						
Mean	20 7	0 39	59	30	92	13
SD	4 58	0 069	12 8	7 8	22 7	14 1
n	20	20	19	20	16	18
200 + 1,000 mg/kg per day						
Mean	21 2	0 38	53	44	107	12
SD	4 25	0 090	14 6	26 4	34 2	7 6
n	19	19	18	17	10	14

TABLE B2
Summary of Clinical Chemistry Parameters for Swiss (CD-1) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations

Female-A Mice

	BUN mg/dL	Crea mg/dL	ALP U/L	ALT U/L	AST U/L	BilA µmol/L
400 + 250 mg/kg per day						
Mean	24 2**	0 40	79	26	76	12
SD	5 62	0 034	41 6	6 4	18 3	4 2
n	12	12	11	11	10	10
400 + 500 mg/kg per day						
Mean	27 8**	0 42	70	24*	72	19
SD	3 35	0 064	14 6	10 3	38 4	7 0
n	12	12	12	12	11	12
400 + 1,000 mg/kg per day						
Mean	24 4**	0 39	56	28	83	12
SD	3 94	0 044	23 4	8 0	23 3	4 5
n	10	9	10	9	9	9

[a] AZT + clarithromycin

* Significantly different (P<0 05) from the control by Dunnett's test

** Significantly different (P<0 01) from the control by Dunnett's test

APPENDIX C
NECROPSY FINDINGS

TABLE C1
Summary of Principal Necropsy Findings for Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations

Male Mice

Dose[a]	0 + 0	200 + 0	400 + 0	0 + 250	0 + 500	0 + 1,000
n[b]	10	10	10	10	10	10
Lesion	**Incidence**					
Stomach, dilatation and/or mottled	0	0	0	0	0	0
Carcass, pale	0	0	0	0	0	0
Kidney, mottled and/or pale	0	0	0	0	0	0
Spleen, small	0	0	0	0	0	0
Thymus, small	0	0	1	0	1	0
Bone, deformity	0	0	0	0	1	1
Dose[a]	200 + 250	200 + 500	200 + 1,000	400 + 250	400 + 500	400 + 1,000
n[b]	10	10	10	10	10	10
Lesion	**Incidence**					
Stomach, dilatation and/or mottled	0	0	0	0	0	2
Carcass, pale	0	0	0	0	0	1
Kidney, mottled and/or pale	1	0	1	0	1	0
Spleen, small	1	0	0	0	0	1
Thymus, small	0	0	2	1	2	3
Bone, deformity	0	0	1	0	0	1

[a] AZT + clarithromycin in mg/kg per day
[b] n = number of animals examined in group

TABLE C1
Summary of Principal Necropsy Findings for Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations

Female-A Mice

Dose[a]	0 + 0	200 + 0	400 + 0	0 + 250	0 + 500	0 + 1,000
n[b]	20	20	20	20	20	20
Lesion	**Incidence**					
Stomach, dilatation and/or mottled	0	0	0	0	0	0
Carcass, pale	0	1	0	0	1	1
Kidney, mottled and/or pale	0	0	0	0	0	2
Liver, enlarged	0	0	0	0	0	1
Spleen, small	0	0	0	0	0	0
Spleen, enlarged	0	0	1	0	0	3
Thymus, small	0	0	0	0	2	1
Bone, deformity	0	0	0	0	1	0
Dose[a]	200 + 250	200 + 500	200 + 1,000	400 + 250	400 + 500	400 + 1,000
n[b]	20	20	20	20	20	20
Lesion	**Incidence**					
Stomach, dilatation and/or mottled	0	1	4	2	2	4
Carcass, pale	0	0	1	1	4	4
Kidney, mottled and/or pale	0	0	2	0	3	1
Liver, enlarged	0	0	0	0	0	0
Spleen, small	0	0	0	0	1	0
Spleen, enlarged	0	0	2	0	0	1
Thymus, small	0	0	2	1	1	1
Bone, deformity	0	0	2	1	1	0

[a] AZT + clarithromycin in mg/kg per day
[b] n = number of animals examined in group

TABLE C1
Summary of Principal Necropsy Findings for Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations

Female-B Mice

Dose[a]	0 + 0	200 + 0	400 + 0	0 + 250	0 + 500	0 + 1,000
n[b]	15	15	16	15	14	15
Lesion	**Incidence**					
Liver, pale	0	0	0	0	0	0
Spleen, enlarged	0	0	0	0	0	0
Bone, deformity	0	0	0	0	0	0
Dose[a]	200 + 250	200 + 500	200 + 1,000	400 + 250	400 + 500	400 + 1,000
n[b]	15	15	14	14	16	15
Lesion	**Incidence**					
Liver, pale	0	0	0	0	1	0
Spleen, enlarged	0	1	1	0	1	4
Bone, deformity	0	0	0	1	0	0

[a] AZT + clarithromycin in mg/kg per day
[b] n = number of animals examined in group

APPENDIX D
MALE REPRODUCTIVE TISSUE EVALUATIONS

TABLE D1
Summary of Male Reproductive Tissue Evaluations in the Reproductive, Developmental, and General Toxicity Studies of AZT and Clarithromycin Combinations in Swiss (CD-1®) Mice

Dose[a]	Left Caudal Weight (g)	Left Epididymal Weight (g)	Left Testicular Weight (g)	Epididymal Sperm Motility (%)
0 + 0	0 0195 ± 0 0012 (10)[b]	0 0476 ± 0 0026 (10)	0 1256 ± 0 0042 (9)	73 56 ± 1 78 (8)
0 + 250	0 0200 ± 0 0009 (10)	0 0498 ± 0 0015 (10)	0 1270 ± 0 0037 (10)	74 50 ± 1 23 (9)
0 + 500	0 0198 ± 0 0009 (10)	0 0490 ± 0 0013 (10)	0 1173 ± 0 0040 (10)	68 48 ± 3 24 (10)
0 + 1,000	0 0195 ± 0 0008 (10)	0 0500 ± 0 0012 (10)	0 1227 ± 0 0060 (10)	68 33 ± 3 02 (10)
200 + 0	0 0209 ± 0 0008 (10)	0 0512 ± 0 0013 (10)	0 1272 ± 0 0062 (10)	65 36 ± 3 40 (8)
200 + 250	0 0208 ± 0 0013 (10)	0 0491 ± 0 0023 (10)	0 1181 ± 0 0071 (10)	60 42 ± 5 92 (9)
200 + 500	0 0196 ± 0 0011 (10)	0 0499 ± 0 0024 (10)	0 1266 ± 0 0059 (10)	63 14 ± 6 51 (9)
200 + 1,000	0 0186 ± 0 0011 (10)	0 0485 ± 0 0019 (10)	0 1221 ± 0 0070 (10)	59 38 ± 6 49 (9)
400 + 0	0 0205 ± 0 0007 (10)	0 0486 ± 0 0015 (10)	0 1148 ± 0 0065 (10)	62 90 ± 4 25 (9)
400 + 250	0 0204 ± 0 0012 (10)	0 0504 ± 0 0016 (10)	0 1205 ± 0 0028 (10)	58 82 ± 5 10 (9)
400 + 500	0 0199 ± 0 0010 (10)	0 0524 ± 0 0015 (10)	0 1225 ± 0 0052 (10)	50 67 ± 7 29 (9)
400 + 1,000	0 0177 ± 0 0011 (10)	0 0470 ± 0 0016 (10)	0 1190 ± 0 0065 (10)	55 48 ± 6 91 (9)

Dose[a]	Sperm per mg Cauda ($\times 10^3$)	Sperm per Cauda ($\times 10^6$)	Spermatids per mg Testis ($\times 10^3$)	Spermatids per Testis ($\times 10^6$)
0 + 0	1016 4 ± 90 79 (9)	20 7 ± 1 65 (9)	248 87 ± 10 64 (9)	25 58 ± 1 14 (9)
0 + 250	1052 5 ± 106 99 (10)	20 4 ± 1 55 (10)	209 57 ± 8 95 (10)	22 48 ± 0 63 (10)
0 + 500	1099 4 ± 91 31 (10)	21 5 ± 1 57 (10)	231 59 ± 11 73 (10)	22 56 ± 1 41 (10)
0 + 1,000	1109 1 ± 83 83 (10)	21 6 ± 1 87 (10)	239 44 ± 21 24 (10)	24 61 ± 2 34 (10)
200 + 0	1102 9 ± 82 75 (10)	23 1 ± 2 14 (10)	234 54 ± 15 88 (10)	25 52 ± 2 40 (10)
200 + 250	1145 2 ± 133 07 (10)	23 2 ± 2 60 (10)	212 45 ± 12 13 (10)	21 40 ± 1 87 (10)
200 + 500	1020 7 ± 58 02 (10)	19 9 ± 1 47 (10)	222 71 ± 18 27 (10)	23 16 ± 2 25 (10)
200 + 1,000	1108 7 ± 71 56 (10)	20 6 ± 1 78 (10)	242 77 ± 17 11 (10)	24 16 ± 1 71 (10)
400 + 0	1149 4 ± 91 09 (10)	23 2 ± 1 64 (10)	256 16 ± 14 88 (10)	23 36 ± 1 33 (10)
400 + 250	1115 5 ± 100 35 (10)	22 6 ± 2 32 (10)	230 70 ± 16 07 (10)	23 44 ± 2 07 (10)
400 + 500	1147 1 ± 87 44 (10)	23 2 ± 2 60 (10)	232 47 ± 15 04 (10)	23 97 ± 1 52 (10)
400 + 1,000	1015 1 ± 45 04 (10)	17 7 ± 0 80 (10)	236 86 ± 10 13 (10)	24 91 ± 1 74 (10)

[a] AZT + clarithromycin in mg/kg per day
[b] All values presented as group mean ± standard error (number of animals providing data)
NOTE A two-way analysis of variance indicates no significant AZT or clarithromycin effect and no significant interaction

Other NIEHS Reports and Publications on the Toxicology of AIDS Therapeutics:

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