National Institute of
Environmental Health Sciences

NIEHS Technical Report on the Reproductive, Developmental, and General Toxicity Study of 3'-Azido-3'-deoxythymidine (AZT) and Rifabutin Combinations (CAS Nos. 30516-87-1 and 7255-06-09) Administered by Gavage to Swiss (CD-1®) Mice

AIDS 04

May 2000

NIEHS AIDS Therapeutics
Toxicity Report
Number 4

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on the Reproductive, Developmental,
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3′-Azido-3′-deoxythymidine (AZT) and Rifabutin Combinations

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Administered by Gavage
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NIB Publication 00-3948
May 2000

U.S. Department of Health and Human Services
Public Health Service
National Institutes of Health

FOREWORD

Infection with human immunodeficiency virus (HTV) 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.

CONTRIBUTORS

This report on the reproductive, developmental, and general toxicity studies of 3′-azido-3′-deoxythymidine (AZT) and rifabutin combinations is based primarily on studies that began in March 1995 and ended in May 1995 at Southern Research Institute, Birmingham, AL.

National Institute of Environmental Health Sciences

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PEER REVIEW

The draft report on the reproductive, developmental, and general toxicity studies of 3'-azido-3'-deoxythymidme (AZT) and rifabutm combinations was evaluated by the reviewers listed below. 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 these studies are appropriate and ensure that this reproductive, developmental, and general toxicity study report presents the experimental results and conclusions fully and clearly. The comments of the reviewers were received and reviewed prior to the finalization of this document. Changes were made such that the concerns of the reviewers have been addressed to the extent possible.

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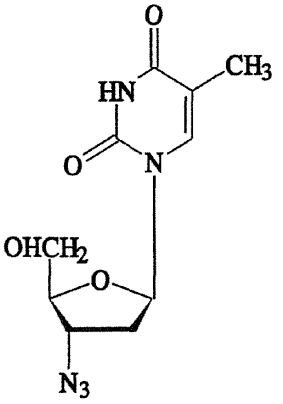
ABSTRACT

3'-Azido-3'-deoxythymidine (AZT) and Rifabutin Combinations



AZT
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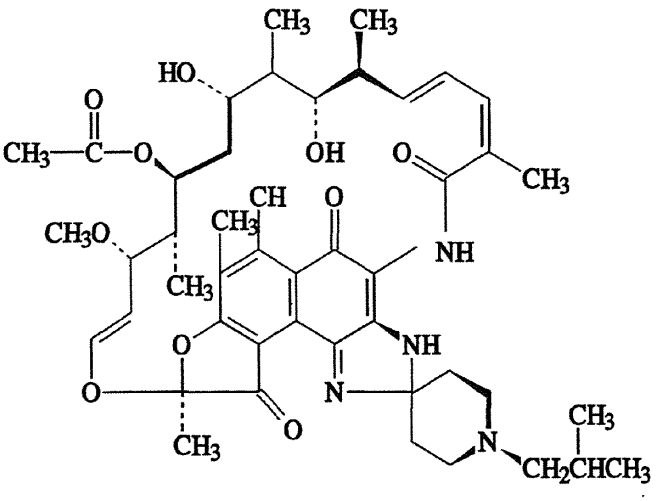
CAS No.: 30516-87-1



Rifabutin
Molecular formula: $C_{46}H_{62}N_4O_{11}$
Molecular weight: 847.02
CAS No.: 7255-06-09

Individual toxicity profiles of AZT and rifabutin in animal models are available. AIDS patients, including pregnant women, may receive combination therapy with these compounds and the toxic potential of AZT and rifabutin combinations in animal models is not known. The purpose of this study was to obtain information on reproductive, developmental and general toxicity of AZT (200 and 400 mg/kg) and rifabutin (80, 320, or 640 mg/kg) combinations in Swiss (CD-1®) mice treated by oral gavage. The doses of AZT were equivalent to 2 and 4 times and the doses of rifabutin were 2, 8, and 16 times the human therapeutic dose, respectively (based on body surface area). Male mice (10 or 15 per group) were dosed from study day 5 until the day prior to sacrifice on study day 24, 25, or 26. Females were divided into two groups designated female-A mice and

female-B mice. The female-A mice (20 per group) were dosed from day 0 to sacrifice for approximately 30 days. They were cohabited with treated males on days 9 through 13 to test for effects on mating behavior, fertilization, and implantation, and caesarean sections were performed on days 28 through 32. The females designated as female-B mice (20 per group) were cohabited with untreated males on days 0 through 4. Sperm-positive female-B mice were dosed during organogenesis on days 6 through 15 of presumed gestation and sacrificed on day 4 of lactation.

Summarized in Table 1 are the significant effects of treatment with AZT and/or rifabutin. Administration of AZT alone resulted in hematopoietic toxicity manifested by mild anemia, leukopenia, and thrombocytosis in male and female mice. In male mice, hematopoietic cell proliferation of the spleen accompanied the anemia.

Administration of rifabutin alone resulted in toxicity in the liver and in the hematopoietic and gastrointestinal systems. The mild anemia evident in male mice treated with rifabutin alone was accompanied by hematopoietic cell proliferation of the spleen and myeloid hyperplasia of bone marrow. A neutrophilic leukocytosis occurred in both males and females. Hepatotoxicity in male and female-A mice was manifested by cytoplasmic alteration and a mixed inflammatory cell infiltrate in the liver. Livers of female-B mice were not examined. The microscopic liver lesions in male and female-A mice were accompanied by elevated liver weights and elevated alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bile acid concentrations. Lesions in the gastrointestinal system consisted of cystic degeneration and inflammation in the glandular stomach of male and female-A mice and epithelial hyperplasia of the forestomach in female-A mice. Mixed inflammatory cell infiltrates were occasionally evident in the lungs of males, and thymic atrophy also occurred in female-A mice treated with rifabutin alone.

Administration of AZT in combination with rifabutin resulted in severe hematotoxicity in male and female-A mice, and the severity of the anemia was far greater than that induced by AZT alone. The anemia caused significant mortality in female-A mice treated for approximately 30 days. The anemia in males was accompanied microscopically by cellular depletion of bone marrow and atrophy of the red pulp of the spleen. Thrombocytosis also occurred in male and female-A mice. A leukopenia with a corresponding decrease in neutrophil counts was evident in both males and in female-A groups in which mortality occurred. Combination therapy also exacerbated the hepatotoxicity induced by rifabutin alone. Mixed cell infiltration and cytoplasmic alteration in the liver of male and female-A mice were accompanied by centrilobular atrophy, which may have been secondary to the severe anemia. Increased ALT activities and elevated bile acid concentrations and liver weights accompanied the microscopic manifestations of liver toxicity. AZT at 400 mg/kg and rifabutin at

640 mg/kg when administered in combination appear to have increased the plasma levels of both compounds as compared to administration of either compound alone.

Degenerative and inflammatory lesions in the stomach of mice treated with the drug combinations were similar to those induced by rifabutin alone. Other lesions encountered consisted of thymic atrophy and atrophy of lymphoid tissue in the spleen and lymph nodes. Mixed inflammatory cell infiltrates in the lungs evident in the males were similar to the lung lesions in mice treated with rifabutin alone and were associated with a reddish-brown pigment that may have represented rifabutin or a metabolite. Mice treated with the drug combinations had significant decreases in body weight. Pallor was commonly observed in female-A mice that died with severe anemia. Other clinical manifestations of toxicity observed in female-A mice that received combination therapy and died consisted of piloerection, decreased motor activity, coldness to the touch, and labored breathing. Many mice treated with rifabutin alone or in combination with AZT developed a transient swelling of the face and had red-colored urine.

Diminished pregnancy rates and diminished mean fetal weight per litter occurred subsequent to treatment with AZT alone and rifabutin alone. Treatment with AZT alone also resulted in an increased number of resorptions and diminished live litter size. Treatment with the combinations resulted in marked decreases in pregnancy rates, live litter size, and mean fetal body weight per litter and an increased number of resorptions. AZT and rifabutin combinations also caused decreases in the number of litters delivered, cumulative survival per litter, and pup weight per litter. Gross external alterations were not evident in the offspring of mice treated with AZT alone, rifabutin alone, or combinations of AZT and rifabutin.

In conclusion, AZT alone caused mild hematopoietic toxicity as indicated by anemia. Rifabutin alone caused mild hematopoietic toxicity, mild liver toxicity, and degenerative and inflammatory lesions in the stomach. Administration of AZT in combination with rifabutin increased the hematopoietic toxicity and anemia caused by either compound alone, contributing to significant mortality of female mice treated for approximately 30 days. AZT increased the hepatic toxicity caused by rifabutin but did not alter the gastric lesions caused by rifabutin. There appears to be an interaction between AZT at 400 mg/kg and rifabutin at 640 mg/kg when administered in combination, causing increases in plasma concentrations of both compounds. AZT alone and rifabutin alone caused reproductive and developmental effects and combination therapy increased these effects. However, when administered alone or in combination, the two compounds did not cause gross external alterations in pups. These results indicate that combination therapy has the potential to markedly increase the general toxicity, especially hematopoietic toxicity.

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

Treatment Regimen	Male Mice	Female-A Mice	Female-B Mice
Body Weights			
AZT alone	No body weight change	Slight decreases in body weights, body weight gains, and gravid uterine weights	No body weight change
Rifabutin alone	No body weight change	Slight decrease in body weight gain in 640 mg/kg group	No body weight change
AZT + rifabutin	Significant decrease in body weight gain in 400 + 640 mg/kg group	Marked decreases in body weights, body weight gains, and gravid uterine weights	Slight decrease in gestational body weights
Clinical Pathology			
AZT alone	Slight decrease in RBC count Mild neutropenia Mild lymphopenia Mild thrombocytosis	Mild anemia Mild neutropenia Mild thrombocytosis	Mild thrombocytosis
Rifabutin alone	Slight decrease in RBC count Increase in reticulocyte count Thrombocytosis Mild neutrophilia Slight increase in ALT activity	Mild neutrophilia Mild lymphocytosis Slight increase in ALT and AST activities and bile acid concentration	Increase in reticulocyte count Mild thrombocytosis
AZT + rifabutin	Severe anemia Thrombocytosis Slight decrease in neutrophil count Mild lymphopenia Slight increase in ALT and SDH activities and bile acid concentration	Severe anemia Decrease in reticulocyte count Mild thrombocytosis Slight decrease in neutrophil count Increase in ALT activity and bile acid concentration	Slight decrease in RBC count Mild thrombocytosis Slight increase in neutrophil count Slight increase in lymphocyte count
Mortality			
AZT alone	Treatment-related mortality not observed	Treatment-related mortality not observed	Treatment-related mortality not observed
Rifabutin alone	Treatment-related mortality not observed	Treatment-related mortality not observed	Treatment-related mortality not observed
AZT + rifabutin	Treatment-related mortality not observed	Treatment-related mortality corresponding with higher dose levels	Treatment-related mortality not observed
Histopathology			
AZT alone	Liver hepatocellular cytoplasmic alteration (slight) Spleen hematopoietic cell proliferation (slight)	Liver hepatocellular cytoplasmic alteration (slight)	No significant alterations

TABLE 1

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

Treatment Regimen	Male Mice	Female-A Mice	Female-B Mice
Histopathology (continued)			
Rifabutm alone	Liver: hepatocellular cytoplasmic alteration (slight), mixed cell infiltration Glandular stomach: cystic degeneration, inflammation Spleen: hematopoietic cell proliferation (slight) Bone marrow: myeloid hyperplasia Lung: mixed cell infiltration	Liver: hepatocellular cytoplasmic alteration (slight), mixed cell infiltration Glandular stomach: cystic degeneration, inflammation Forestomach: epithelial hyperplasia Thymus: atrophy (slight)	No significant alterations
AZT + rifabutin	Liver: hepatocellular cytoplasmic alteration (prominent), mixed cell infiltration, centnlobular atrophy Forestomach: epithelial hyperplasia, ulceration, inflammation Glandular stomach: cystic degeneration, chronic active inflammation Bone marrow: cellular depletion Spleen: red pulp atrophy, lymphoid follicle atrophy Lung: mixed cell infiltration Thymus: atrophy	Liver: hepatocellular cytoplasmic alteration (prominent), mixed cell infiltration, centnlobular atrophy, centnlobular necrosis Forestomach: epithelial hyperplasia, ulceration, inflammation Glandular stomach: cystic degeneration, inflammation Thymus: atrophy	No significant alterations
Reproductive/Developmental			
		Fetuses	**Pups**
AZT alone		Decreases in pregnancy rate, live litter size, mean fetal body weight per litter, increased number of resorptions	No adverse effects
Rifabutin alone		Decreases in pregnancy rate and mean fetal body weight per litter (640 mg/kg group only)	No adverse effects
AZT + rifabutin		Marked decreases in pregnancy rate, live litter size, and mean fetal body weight per litter, increased number of resorptions	Decreases in number of delivered litters, cumulative survival per litter, litter size, and pup weight per litter

INTRODUCTION

AIDS is a lethal multi-system disease that has become a major public health problem since its recognition in 1981 (Gottlieb *et al.*, 1981; Masur *et al.*, 1981; Siegal *et al.*, 1981). The etiological agent of AIDS is a retrovirus now referred to as 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 hematologic results and included lymphoid depletion, reticuloendothelial hyperplasia in spleen and thymus, and bone marrow hypocellularity.

In a 14-week subchronic study (NTP, 1999), B6C3F_1 mice were treated with 0, 25, 50, 100, 400, or 1,000 mg AZT/kg body weight in 0.5% methylcellulose by gavage twice daily. On day 5, statistically significant dose-related decreases were observed in reticulocyte counts in both 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 92 days 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 that affected AZT-treated B6C3F_1 mice was a darkening of the skin on the tail, feet, and/or muzzle.

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 post-dosing were 61 μg/g, or 76 times the antiviral inhibitory dose for 50% of the viral population being tested (ID_{50}). Rabbits were orally dosed 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 post-dosing 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, food consumption, fertility, hematologic parameters, or growth or 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 C_3H/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.

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). Survival of AIDS patients with MAC infection is half that of nonMAC-infected patients. Existing treatment regimens are not consistently successful, and relapses are common. Rifabutin, a rifamycin analogue, has shown significant activity against MAC *in vitro* and *in vivo*.

The pathogenic agent MAC, a ubiquitous organism, 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; disseminated disease was rare, even in severely

immunocompromised patients. Infection occurs via respiratory or gastrointestinal routes, and the gastrointestinal tract is thought to be the most common site of colonization and dissemination.

For reasons not understood, AIDS patients are uniquely predisposed to development of disseminated MAC disease. Fifteen to fifty percent of HIV-infected patients are infected with MAC, which is characterized by fever, diarrhea, night sweats, weight loss, anemia, abdominal pain, and elevated serum alkaline phosphatase. MAC is associated with advanced HIV infection; patients with CD4 lymphocyte counts less that $100/mm^3$ are considered to have increased risk for MAC. Other factors that correlate with the development of disseminated disease are previous opportunistic infection, an interruption in AZT therapy, and significant anemia. 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).

Until recently, available antimicrobial drugs were not active against MAC (Masur, 1993). The intracellular location of this organism serves to protect it from drugs that do not penetrate the plasma membrane. Recently, several new antimicrobial agents have been developed including rifabutin, the first drug approved by the FDA targeted against this organism. Rifabutin, 4-deoxo-3,4-[2-spiro-(N-isobutyl-4-piperidyl)](1H)-imidazole (2,5-dihydro)-rifamycin S, is a semisynthetic spiropiperidyl derivative of rifamycin S.

Following oral administration in humans, rifabutin is rapidly but incompletely absorbed; measurable amounts of the drug are detected in the serum within 30 minutes. Peak serum levels occur 4 hours after administration. The half-life in serum is 16 hours, with detectable levels still present at 24 hours after ingestion.

Two hours after an oral dose of rifabutin in rats, the highest concentrations were found in the liver, followed by lung, abdominal adipose tissue, and spleen (Battaglia *et al.*, 1991). Seventy-two hours after administration, the sequence was abdominal adipose tissue, liver, spleen, bone marrow, and lung.

Pharmacokinetic parameters of rifabutin in healthy male volunteers given 28 doses of 300 to 1,200 mg/day were reported by Skinner *et al.* (1989). The plasma concentration data were consistent with a two-compartment open model with a terminal half-life of 36 hours. Following the 1,200 mg dose, peak and trough plasma levels were 907 and 194 ng/mL, respectively.

^{14}C-Rifabutin is eliminated by renal and fecal routes (Battaglia *et al.*, 1991); the renal route appears to predominate in humans (50.19%) and monkeys (46.73%), whereas fecal excretion is the major elimination route in rats (48.09%) and rabbits (45.01%). Peak plasma levels occurred in 1 to 4 hours in these species.

Significant levels of 31-OH rifabutin were detected by 8 to 24 hours in all four species. The 25-O-deacetyl metabolite was detected in rats and humans only. Polar metabolites were also detected. Rifabutin was excreted unchanged in urine in the rat (8.5%) and in humans (4.6%); only trace amounts were found in rabbit and monkey urine.

Few reports on the toxicity of rifabutin in animals are available. Efficacy studies did not include adverse effects of the drug at the concentrations tested. However, the *Physician's Desk Reference* (1995) does include the following: increased bilirubin and liver weight have been reported in rats, mice, and monkeys given 5, 6, and 8 times the human daily dose. Testicular atrophy was seen in baboons and rats at four and 40 times the recommended human dose, respectively.

Rifabutin causes multinucleated hepatocytes in rats (Scampini *et al.*, 1993). Multinucleated hepatocytes were dose- and gender-related and appeared predominantly in male rats after 5 weeks of treatment. This effect is unique to the rat, not caused by rifampin (a derivative of rifabutin), and not associated with an increased number of liver tumors.

In a clinical study of the antiviral activity of rifabutin, Siegal *et al.* (1990) reported a reversible syndrome of arthralgia/arthritis in patients receiving doses that exceeded 1,050 mg/day; aphthous stomatitis and uveitis were seen at doses above 1,800 mg/day. Doses less than 1 g/day were well tolerated in patients with AIDS-related complex. An orange-tan skin pigmentation and discoloration of body fluids were noted in almost all patients. Other adverse effects of rifabutin include neutropenia, thrombocytopenia, rash, nausea, flatulence, serum aminotransferase elevations, and myositis.

The myelotoxicity of rifabutin in combination with AZT *in vitro* was investigated in anticipation of a synergistic effect of these drugs (Volpe *et al.*, 1993). While both AZT (5 μM) and rifabutin (5 μM) alone were moderately toxic to hematopoietic progenitors and slightly toxic to granulocyte-macrophage precursors, the combination of these drugs did not exceed the toxicity of AZT alone.

STUDY RATIONALE

The objective of this study was to obtain controlled laboratory data on the general and reproductive and developmental toxicity of AZT and rifabutin when administered in combination to mice. Male and female mice were administered AZT (200 or 400 mg/kg) alone, rifabutin (80, 320, or 640 mg/kg) alone, or combinations of AZT and rifabutin by gavage. Mice were treated twice daily, approximately 6 hours apart. Adult mice were evaluated for mortality, clinical signs, body weight, sperm function, pathology, plasma drug levels, and clinical pathology parameters. Offspring were evaluated for viability, external abnormalities, and weight.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF CHEMICALS

3′-Azido-3′-deoxythymidine (AZT; lot 7494-36-05) was manufactured by Raylo Chemicals (Edmonton, Alberta) and Burroughs Wellcome (Research Triangle Park, NC) and supplied as a powder. Rifabutin (lot 4041M155) was manufactured by Pharmacia, Inc. (Piscataway, NJ).

Rifabutin, a violet-red crystalline powder, is stable at room temperature. It is minimally soluble in water, slightly soluble in ethanol, soluble in methanol, and highly soluble in chloroform (O'Brien *et al.*, 1987).

The purity of AZT was determined to be greater than 99% by high-performance liquid chromatography. Purity of the rifabutin used in this study was estimated by high-performance liquid chromatography to be approximately 99%. Infrared and nuclear magnetic resonance spectroscopy analyses performed on AZT and rifabutin produced spectra which were consistent with the structures of AZT and rifabutin and agreed with the reference spectra. The vehicle used in this study was an aqueous solution containing 0.2% methylcellulose and 0.1% Tween 80.

DOSE FORMULATIONS

The required amounts of AZT and rifabutin were combined with the required amount of the vehicle. The dose formulation was then stirred until visually homogeneous. A stability study conducted on AZT/rifabutin stored at 4° C indicated no significant loss of either test chemical for at least 33 days. Dose formulations were stored refrigerated, protected from light, and used within 20 days.

Samples at each dose concentration from the initial mix were analyzed prior to dosing. Residual formulations taken from the same mix after dosing were also analyzed. Prior to dosing, the concentrations of AZT ranged from 100% to 106% of the target concentrations. The concentrations of rifabutin were 104% to 111% of the target concentrations. Concentrations of AZT and rifabutin in the residual formulations ranged from 96% to 104% and 91.6% to 156% of the target concentrations, respectively.

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 five males or five females per cage during quarantine, uniquely identified before randomization, and individually housed after randomization, except during cohabitation.

The mice were housed in polycarbonate cages with solid bottoms and sides. Average temperature in the animal rooms was 70.7° F, with a standard deviation of 0.7° F; average relative humidity was 47.2%, with a standard deviation of 6.8%.

At terminal sacrifice, blood samples were collected from five male and five female sentinel animals as part of the animal disease screening program. Results indicated all animals were free of viral antibodies.

AIDS patients receive combination therapy with rifabutin and AZT, and 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 (CD-1®) mouse chosen for this study is one of the mouse models routinely used for reproductive and developmental toxicity studies by the NIEHS.

The basic premise for dose concentration selection is that the high dose concentration should induce some measurable evidence of toxicity (e.g., anemia, weight loss, target organ toxicity). In a number of reproductive, developmental, and toxicity studies under this project, AZT at the doses selected (200 and 400 mg/kg) caused resorptions and decreased litter sizes. The human therapeutic dose is 10 mg/kg per day (*PDR*, 1995). The selected doses are 20- and 40-times human doses, but on a body surface area basis (Freirich *et al.*, 1966), the doses are close to two and four times the therapeutic dose. Recent studies under this project have also demonstrated dose-related decreases in hematologic parameters at these doses.

Dose concentrations selected for rifabutin (80, 320, and 640 mg/kg per day) are 2, 8, and 16 times the therapeutic dose and were based upon the following: the therapeutic dose in the mouse for *Mycobacterium avium* complex (MAC) infection is 20 to 40 mg/kg per day for up to 60 days (Orme, 1988; Saito *et al.*, 1988); no toxicity was reported at these doses. The clinical dose for MAC prophylaxis in humans is 4.3 mg/kg per day (300 mg per day; Nightingale *et al.*, 1993), equivalent to 53 mg/kg per day in the mouse. The maximum tolerated dose reported in humans is 14.2 mg/kg per day (1,000 mg per day; Siegal *et al.*, 1990), equivalent to 175 mg/kg per day in the mouse.

The design of this study is a modification of a design published elsewhere (Harris *et al.*, 1992). A brief summary of the study design is provided in Table 2. The oral route of administration was selected because it is the route used in humans, and the study was conducted in Swiss (CD-1®) mice because this strain is routinely used for reproductive and developmental toxicity evaluations. Combinations of AZT and rifabutin were administered by gavage as a single formulation in an aqueous solution containing methylcellulose and Tween 80. Total daily doses of 20 mL/kg were divided into two equal doses of 10 mL/kg given approximately 6 hours apart. Mice were divided into three groups as follows:

Male Mice: Ten males were assigned to each dose group. Five additional males were added to selected groups for determination of plasma concentrations of the test compounds. Prior to dosing, male mice were cohabited with female-B mice on study days 0 to 4. Males were dosed from study day 5 to the day prior to sacrifice for 20 days. Males were cohabited with female-A mice on study days 9 to 13 to identify 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 euthanatized with CO_2, necropsy was conducted, and the testis and epididymides were collected and prepared for evaluation of sperm parameters as described in the Sperm Function Evaluation section of this report.

Female-A Mice: Twenty females were assigned to each dose group. Female-A mice were dosed from study day 0 to the day prior to sacrifice for approximately 30 days. Male mice were cohabited with female-A mice on study days 9 to 13 to identify effects of treatment on mating behavior, fertilization, implantation, or the initial stages of development. 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, 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. The 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 uteri of all sperm-negative females were examined for evidence of unsuccessful pregnancy and then press-plated between two heavy plates of glass to visualize implantation sites. Additional endpoints for all female-A mice included gravid uterine weight and number of implantation sites, resorptions, corpora lutea, and dead and live fetuses.

Female-B Mice: Twenty females were assigned to each dose group. Prior to dosing, female-B mice were cohabited with males on study days 0 to 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 evenly across dose groups prior to gestation day 6. Female-B mice were subsequently dosed during gestation days 6 to 15 (for 10 days), during the fetal organogenesis period, to identify effects on fetal development. Residual effects on parturition and the beginning of lactation were also evaluated. Beginning on 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 of completed delivery was determined to the nearest day and designated as postnatal day 0. On postnatal days 0 and 1, dam body weights were recorded along with the number of live and dead pups, the number of male and female pups, the incidence of any gross malformations, and live pup weights. Dead pups were discarded. On postnatal day 4, all female-B mice, including any that did not deliver, were weighed, and blood samples were collected from the retroorbital sinus for hematology evaluations. These mice were then euthanatized with CO_2, and complete necropsies were performed. The uterus was removed and press-plated. All pups were weighed and given a thorough external examination for lesions and malformations, and the gender was recorded. The pups were then euthanatized with CO_2 and preserved in Bouin's fixative.

Clinical Pathology

All blood samples were collected from the retroorbital sinus under CO_2:O_2 (70/30) anesthesia. Blood for hematology analyses was collected in a tube containing EDTA, and blood for clinical chemistry analyses was collected in a tube without anticoagulant. Animals were selected in random order for blood collection, and samples were analyzed in the order collected.

Erythrocyte, platelet, and leukocyte counts; hematocrit; hemoglobin concentration; mean cell hemoglobin; mean cell volume; mean cell hemoglobin concentration; and leukocyte differentials 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 erythrocyte and platelet morphology if necessary. Sorbitol dehydrogenase, total bile acids, alanine aminotransferase, alkaline phosphatase, and aspartate aminotransferase were determined using the Roche Cobas Fara™ automated analyzer. Priority for clinical chemistry tests was assigned in the order listed above.

Blood (approximately 0.6 mL) was collected by retroorbital puncture into heparinized tubes at 15, 30, 60, and 90 minutes following the last dose for determination of AZT and rifabutin levels in plasma. Two mice per

selected dose group (male and female-A mice) were bled at each time point when possible. Dose groups designated to be bled for plasma concentration determinations included females that received 80 mg/kg rifabutin with or without 400 mg/kg AZT and males and females that received 320 or 640 mg/kg rifabutin with or without 400 mg/kg AZT.

Males used for plasma concentration determinations included extra mice (five per dose group) from the shipment of mice for this study, as well as 3 of 10 males from selected dose groups that were on study; the remaining males in each dose group were bled for clinical pathology determinations. Female mice bled for plasma concentration determinations were pregnant female-A study mice, when possible.

Sperm Function Evaluation

Sperm motility was evaluated at necropsy in the following manner. The left epididymis was isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis). Modified Tyrode's buffer was applied to slides, and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers.

Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered neutral saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution. Sperm density was then determined microscopically with the aid of 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. Homogenization-resistant spermatid nuclei were counted with a hemocytometer.

Histopathology

Necropsy was performed on all animals (except sperm-negative female-B mice). The liver of male and female-A mice was weighed. Histopathology of male and female-A mice was conducted on the tissues listed in Table 2. With the exception of the testes and epididymides, which were fixed in Bouin's and stained with PAS, all other tissues were fixed in 10% neutral buffered formalin. These tissues were then trimmed to a maximum thickness of 0.3 cm for processing, embedded in paraffin, sectioned at 4 to 6 μm thickness, stained with hematoxylin and eosin, and examined by light microscopy.

TABLE 2
Experimental Design and Materials and Methods in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin 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
17 days

Age When Placed on Study
88 days

Duration of Dosing
Male mice day 5 to day prior to sacrifice (except for those animals designated for plasma compound level determination)
Female-A mice day 0 to day prior to sacrifice (except for those animals designated for plasma compound level determination)
Female-B mice gestation days 6 to 15

Days of Cohabitation
Male and Female-A mice days 9 to 13
Male and Female-B mice days 0 to 4
When possible, one male and two females within the same dose group were housed together by consecutive animal number

Necropsy Dates
Male mice days 25 and 26
Female-A mice days 28 to 32
Female-B mice day 5 (sperm-negative), days 24 through 29 of presumed gestation (sperm-positive, not delivered), postnatal day 4 (sperm-positive, delivered)

Average Age at Necropsy
Male mice 113-114 days
Female-A mice 116-120 days
Female-B mice 112-117 days

Size of Study Groups
Male mice 10 or 15
Female-A mice 20
Female-B mice 20

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

Animals per Cage
One animal per cage, except during cohabitation

Method of Animal Identification
Tail tattoo

Diet
NIH-07 pelleted feed, available *ad libitum*

TABLE 2
Experimental Design and Materials and Methods in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin in Swiss (CD-1®) Mice

Water
Tap water (Birmingham, AL), available *ad libitum*

Cages
Polycarbonate cages with solid bottoms and sides

Bedding
Heat-treated hardwood chips (Sani-Chips®, P J Murphy Forest Products Corporation, Montville, NJ)

Cage Filters
Remay® spun-bonded polyester (Andico, Birmingham, AL)

Racks
Stainless steel (Lab Products, Maywood, NJ)

Animal Room Environment
Temperature 70 7° ± 0 7° F
Relative humidity 47 2% ± 6 8%
Fluorescent light 12 hours fluorescent light/day
Room air minimum of 10 changes/hour

Doses
Daily doses in methylcellulose and Tween 80 by gavage
0 mg AZT + 0 mg rifabutin per kg body weight per day
0 mg AZT + 80 mg rifabutin per kg body weight per day
0 mg AZT + 320 mg rifabutin per kg body weight per day
0 mg AZT + 640 mg rifabutin per kg body weight per day
200 mg AZT + 0 mg rifabutin per kg body weight per day
200 mg AZT + 80 mg rifabutin per kg body weight per day
200 mg AZT + 320 mg rifabutin per kg body weight per day
200 mg AZT + 640 mg rifabutin per kg body weight per day
400 mg AZT + 0 mg rifabutin per kg body weight per day
400 mg AZT + 80 mg rifabutin per kg body weight per day
400 mg AZT + 320 mg rifabutin per kg body weight per day
400 mg AZT + 640 mg rifabutin per kg body weight per day

Type and Frequency of Observation
Mortality/moribundity twice daily
Clinical findings once daily
Vaginal plugs days 10 to 14 for female-A mice, days 1 to 5 for female-B mice
Body weights days 3, 5, 9, 13, 17, 21, 23, and sacrifice for male mice, days 0, 4, 12, 16, 20, 23, 26, and sacrifice for female-A mice, gestation days 0, 8, 12, 15, and postnatal days 0, 1, and 4 for female-B mice, postnatal days 0, 1, and 4 for F_1 pups

Method of Sacrifice
Carbon dioxide asphyxiation

Necropsy
Complete necropsies were performed on all breeder animals except sperm-negative female-B mice Liver was weighed for male and female-A mice

Histopathology
Histopathology was performed on all male and female-A mice In addition to gross lesions and associated lymph nodes, the following tissues were examined **Male mice** - brain, bone marrow, epididymis, heart, kidneys, liver, lungs, lymph nodes (mandibular and mesenteric), spleen, stomach, and thymus **Female-A mice** - liver and stomach Pups were examined externally only

TABLE 2
Experimental Design and Materials and Methods in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin in Swiss (CD-1®) Mice

Clinical Pathology
Hematology (all animals) and clinical chemistry (male and female-A mice) evaluations were conducted at terminal sacrifice, except for those designated for plasma AZT and rifabutin level determinations
Hematology · erythrocyte, reticulocyte, and platelet counts, hematocrit, hemoglobin, mean cell hemoglobin, mean cell volume, mean cell hemoglobin concentration, leukocyte counts and differentials, erythrocyte and platelet morphology (if necessary)
Clinical Chemistry sorbitol dehydrogenase, alanine aminotransferase, alkaline phosphatase, and aspartate aminotransferase activities and bile acid concentrations of male and female-A mice
Plasma AZT and Rifabutin Levels
Selected animals from selected dose groups (male and female-A mice only) were bled for plasma AZT and rifabutin level determinations
Sperm Motility Evaluation
Sperm samples were collected from all males at terminal sacrifice The parameters evaluated included spermatid heads, spermatid counts, and motility The left epididymis and cauda epididymis were weighed

STATISTICAL METHODS

Paternal and maternal body weights were analyzed using Bartlett's test of homogeneity of variances and the analysis of variance (Snedecor and Cochran, 1967a). If Bartlett's test was not significant ($P > 0.05$) and the analysis of variance was significant ($P \leq 0.05$), then Scheffe's test (Scheffe, 1953) was used to identify the statistical significance of individual groups. If Bartlett's test was significant ($P \leq 0.05$), the Kruskal-Wallis test (Sokal and Rohlf, 1969) was used; in cases in which the Kruskal-Wallis test was significant ($P \leq 0.05$), 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. For F_0 generation sires and dams, the analysis of covariance (Snedecor and Cochran, 1967b) was used to evaluate mean body weight changes and mean maternal body weight changes. Observations for delivered and dead conceptuses of the female-A dams and for fetuses from female-A dams caesarean-sectioned on an estimated day 14 of gestation 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 final mean body and epididymis weights. Epididymis-weight-to-body-weight ratios were also calculated. Final mean body weights of males and females and mean epididymis weights and epididymis-weight-to-body-weight ratios for males for each dosed group were compared to those of the control group by a two-tailed Student's *t*-test. The standard deviations used in the *t*-tests were obtained by pooling the individual values for the vehicle control and dosed groups. Hematology and clinical chemistry data were evaluated using Dunnett's (1955) test.

Severity grades for lesions in liver, spleen, thymus, stomach, and bone marrow were analyzed for interaction using two-way analysis of variance. If a significant interaction between AZT and rifabutin was observed, mean values were plotted to graphically display the interaction between the two chemicals. In the absence of interaction, vehicle control and dosed group means were compared using either Williams' (Williams, 1971, 1972) or Dunnett's (Dunnett, 1955) multiple comparison procedures. The choice between the two tests was based on evidence of a dose-related trend in the data as determined by Jonckheere's test (Jonckheere, 1954). Williams' test was applied if there was an indication of trend ($P \leq 0.01$), and Dunnett's test was used in the absence of a trend. In selected cases in which interaction was found to be significant, the multiple comparison tests described above were used to detect treatment-related effects.

Proportion data (e.g., clinical findings and incidences of pregnancy, resorption, death, and total resorption) for mice presumed pregnant were analyzed using the Cochran-Armitage test for a linear trend in proportions (Snedecor and Cochran, 1967c) and Fisher's exact test (Siegel, 1956). Epididymal sperm motility data were analyzed by the Wilcoxon rank sum test for pairwise nonparametric comparisons (Wilcoxon, 1945).

RESULTS AND DISCUSSION

SURVIVAL AND CLINICAL FINDINGS

Male Mice

No male mice died before scheduled sacrifice. All male mice that received 320 or 640 mg/kg rifabutin alone or in combination with 200 or 400 mg/kg 3′-azido-3′-deoxythymidine (AZT) had red-colored urine; five of ten male mice in these groups had swollen faces. Piloerection was noted in male mice administered 0 + 640 mg/kg (5 of 10), 200 + 640 (5 of 10), or 400 + 640 mg/kg (6 of 10). The only other clinical finding noted in any male mice was red-colored urine in two mice each in the 0 + 80 and 200 + 80 mg/kg groups.

Female-A Mice

Fifty-five female-A mice died prior to scheduled sacrifice (Table 3). Five were administered 200 + 320 mg/kg, 20 were administered 200 + 640 mg/kg, 10 were administered 400 + 320 mg/kg, and the remaining 20 were administered 400 + 640 mg/kg. These mice died on study days 4 through 27. In general, mice considered moribund had one or more of the following signs: coldness to the touch, decreased motor activity, labored breathing, tremors, pallor, and hindlimb dragging.

Red-colored urine occurred in all female-A mice administered 320 or 640 mg/kg rifabutin alone or in combination with 200 or 400 mg/kg AZT, three mice administered 200 or 400 mg/kg AZT + 80 mg/kg rifabutin, and all mice administered 80 mg/kg rifabutin alone. Piloerection occurred in 11 of 20 mice administered 200 + 320 mg/kg, all mice administered 400 + 320 mg/kg, and all mice administered 640 mg/kg rifabutin alone or in combination with 200 or 400 mg/kg AZT. Swollen faces were observed in 10 of 20 mice in each of the groups administered 80, 320, or 640 mg/kg rifabutin alone or 320 or 640 mg/kg rifabutin in combination with 200 or 400 mg/kg AZT.

TABLE 3
Early Deaths in Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations

Dose[a]	Number of Deaths	Day of Death	Type of Death	Clinical Findings
200 + 320	4	4-26	Moribund	Swollen face, red-colored urine, dragging hindlimbs, piloerection, decreased motor activity, coldness to the touch, pallor, labored breathing
	1	27	Found dead	Swollen face, red-colored urine
200 + 640	20	14-22	Moribund	Swollen face, red-colored urine, piloerection, decreased motor activity, coldness to the touch, labored breathing
400 + 320	10	19-27	Moribund	Swollen face, red-colored urine, piloerection, decreased motor activity, coldness to the touch, pallor, labored breathing
400 + 640	19	6-22	Moribund	Swollen face, red-colored urine, piloerection, decreased motor activity, coldness to the touch, pallor, labored breathing, tremors
	1	11	Found dead	Swollen face, red-colored urine, piloerection

[a] Daily gavage doses of AZT + rifabutin (mg/kg per day)

Female-B Mice

No female-B mice died before the scheduled sacrifice. Red-colored urine was observed during gestation and lactation in almost all mice administered 320 or 640 mg/kg rifabutin alone or in combination with 200 or 400 mg/kg AZT. Red-colored urine also occurred in one mouse that received 400 + 80 mg/kg. Swollen faces were observed during gestation in six mice administered 320 mg/kg rifabutin alone; in three mice administered 640 mg/kg rifabutin alone; in four and three mice administered 200 mg/kg AZT + 320 or 640 mg/kg rifabutin, respectively; and in three and four mice administered 400 mg/kg AZT + 320 or 640 mg/kg rifabutin, respectively.

BODY AND ORGAN WEIGHTS

Male Mice

Administration of 400 + 640 mg/kg caused a significant decrease ($P \leq 0.01$) in mean body weight gain (Figure 1) for the entire dosing period (days 5 to 23). AZT alone (200 or 400 mg/kg), rifabutin alone (80, 320, or 640 mg/kg), or 200 or 400 mg/kg AZT + 80 or 320 mg/kg rifabutin did not affect mean body weights or body weight gains during the dosing period. No other statistically significant differences ($P \leq 0.05$) occurred.

In males, significant dose-related elevations in absolute liver weights were noted (Figure 2 and Table B1). Male mice treated with 640 mg/kg rifabutin alone, 200 mg/kg AZT + 320 or 640 mg/kg rifabutin, or 400 + 320 mg/kg had increased ($P \leq 0.05$) absolute liver weights. These elevations correlated well with histopathologic and clinical chemistry alterations indicative of liver toxicity.

Female-A Mice

Mice administered 200 or 400 mg/kg AZT alone had mean body weights (Figure 3) significantly reduced ($P \leq 0.05$ or $P \leq 0.01$) compared to the vehicle control group mean on study day 26 and at termination. Mean body weight gains were significantly reduced ($P \leq 0.05$ or $P \leq 0.01$) for the 200 and 400 mg/kg AZT groups on study days 0 to 26 compared to the vehicle control group. These decreased mean body weights resulted from reduced gravid uterine weights ($P \leq 0.01$) in the 200 and 400 mg/kg AZT groups. When gravid uterine weights were subtracted, the final mean body weights were comparable to the vehicle control group value.

Administration of rifabutin alone at doses as high as 640 mg/kg did not affect mean body weights, mean body weight gains, final mean body weights, or gravid uterine weights of female-A mice. No significant ($P \leq 0.05$) differences in mean body weights occurred between this group and the vehicle control group. A significant decrease ($P \leq 0.01$) in mean body weight gain occurred in the 640 mg/kg rifabutin group on study days 23 to 26.

Female-A mice administered 200 mg/kg AZT + 320 or 640 mg/kg rifabutin had reduced and/or significantly reduced ($P \leq 0.05$ or $P \leq 0.01$) mean body weights on study days 16 (200 + 640 mg/kg group only), 20, 23, 26, and at termination (for surviving mice). A similar pattern occurred in groups treated with 400 mg/kg AZT + 80, 320, or 640 mg/kg rifabutin. These differences reflected reduced gravid uterine weights that occurred in all groups administered both test articles and that survived to caesarean section. The mean corrected final body weights (final body weight minus gravid uterine weight) were comparable among all groups with surviving mice administered both test articles. The exception was reduced body weight of the one mouse

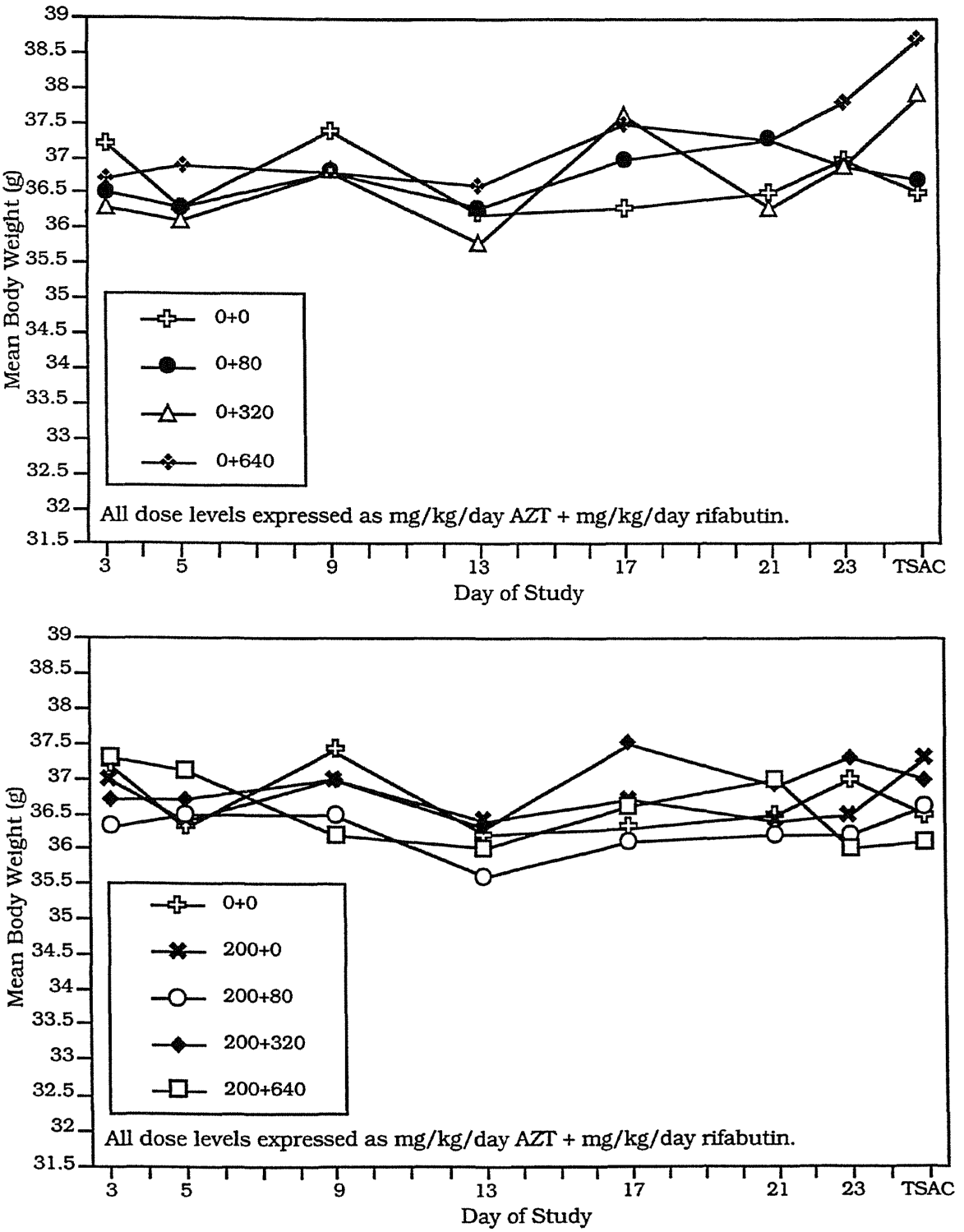


FIGURE 1
Mean Body Weights of Male Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations (TSAC=terminal sacrifice)

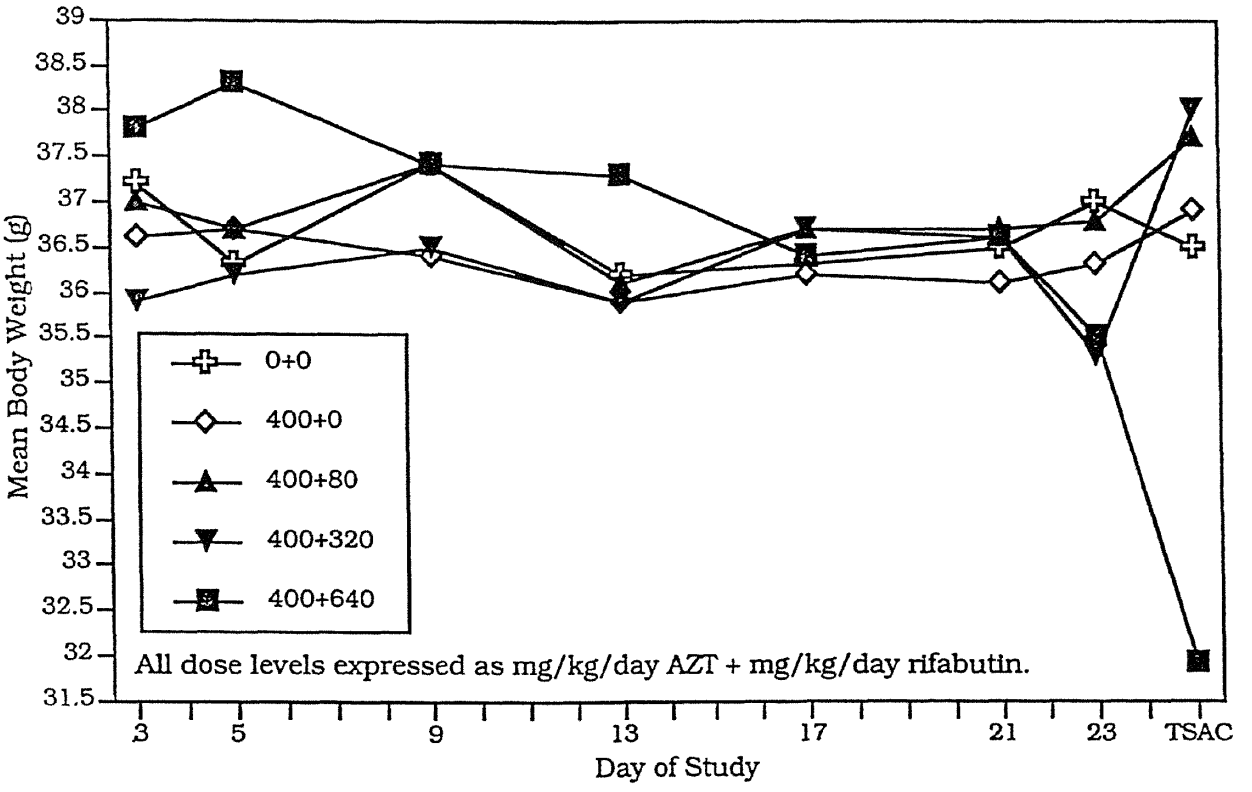


FIGURE 1
Mean Body Weights of Male Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations (TSAC=terminal sacrifice)

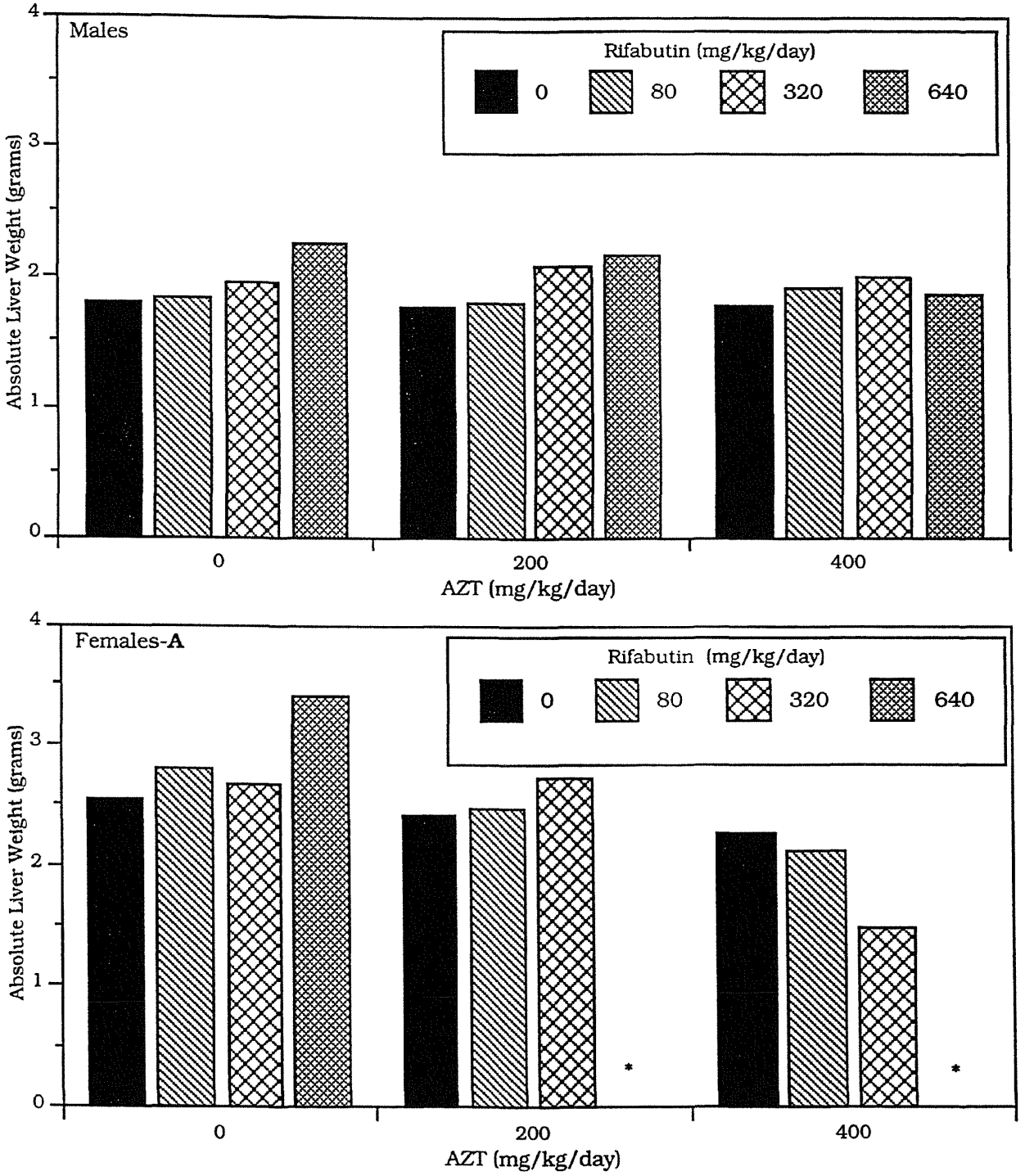


FIGURE 2
Mean Absolute Liver Weights for Male and Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations (*=data not available due to mortality. For variance and statistically significant differences, see Table B1.)

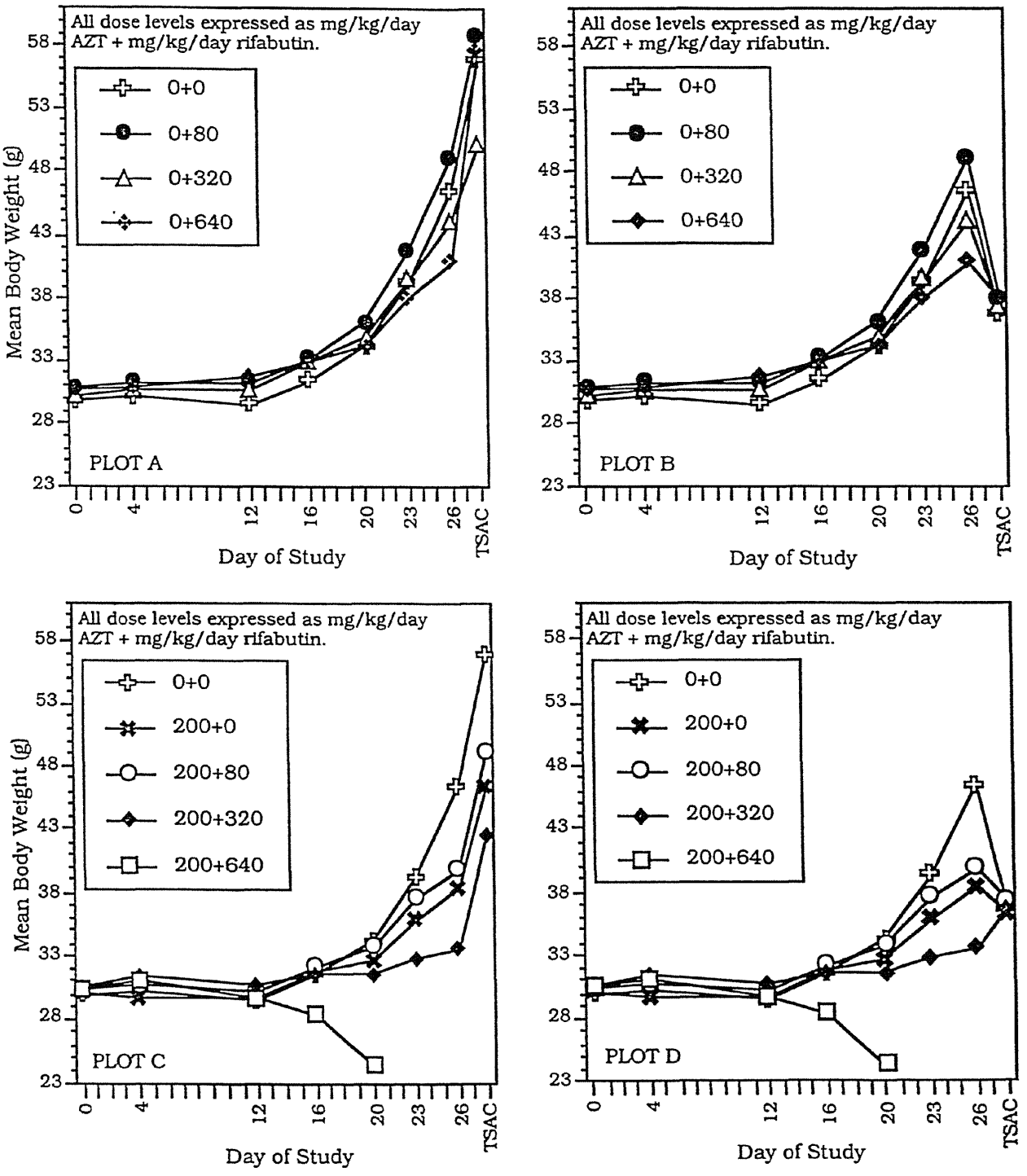


FIGURE 3

Mean Body Weights of Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin 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 final mean body weights and corrected 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.)

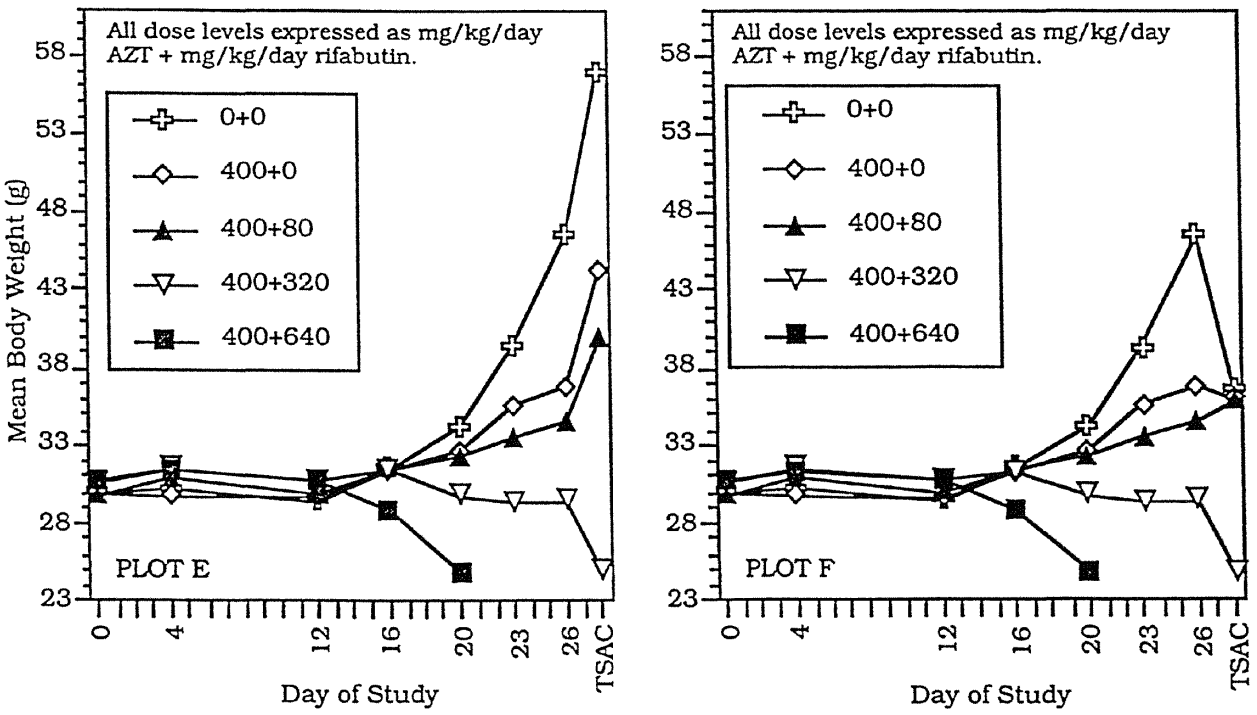


FIGURE 3

Mean Body Weights of Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin 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 final mean body weights and corrected 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.)

in the 400 + 320 mg/kg group that was pregnant and subjected to caesarean section on presumed day 19 of gestation. (All other mice in this group were not pregnant or were sacrificed before day 19 of gestation.)

Mean body weight gains were significantly reduced ($P \leq 0.01$) for the female-A mice administered 200 + 80 mg/kg for study days 23 to 26, 200 + 320 mg/kg on study days 0 to 26, or 200 + 640 mg/kg on study days 0 to 20. Mean body weight gains were also significantly reduced ($P \leq 0.05$ or $P \leq 0.01$) for the female-A mice administered 400 mg/kg AZT in combination with 80 or 320 mg/kg rifabutin on study days 0 to 26 and in combination with 640 mg/kg rifabutin on study days 0 to 20.

Significantly increased ($P \leq 0.05$) absolute liver weights were noted (Figure 2 and Table B1) in female-A mice receiving 640 mg/kg rifabutin alone.

Female-B Mice

Mean gestational body weights (Figure 4) were comparable among the groups administered AZT alone, rifabutin alone, or AZT in combination with rifabutin. The exception was the 400 + 640 mg/kg group, which had significantly reduced ($P \leq 0.01$) mean body weights on gestation day 15 compared to the vehicle control group. Mean gestational body weight gains were significantly reduced ($P \leq 0.05$ or $P \leq 0.01$) for the groups receiving 200 + 640 mg/kg or 400 + 320 mg/kg on days 12 to 15 of gestation and 400 + 640 mg/kg on days 8 to 15 of gestation.

Mean body weights and body weight gains of the treated groups during the lactation period did not significantly ($P > 0.05$) differ from any of the vehicle control group values, although these values tended to be reduced in the group that received 200 + 640 mg/kg. However, this group had only one mouse that delivered pups and the dam and pups survived to lactation day 4.

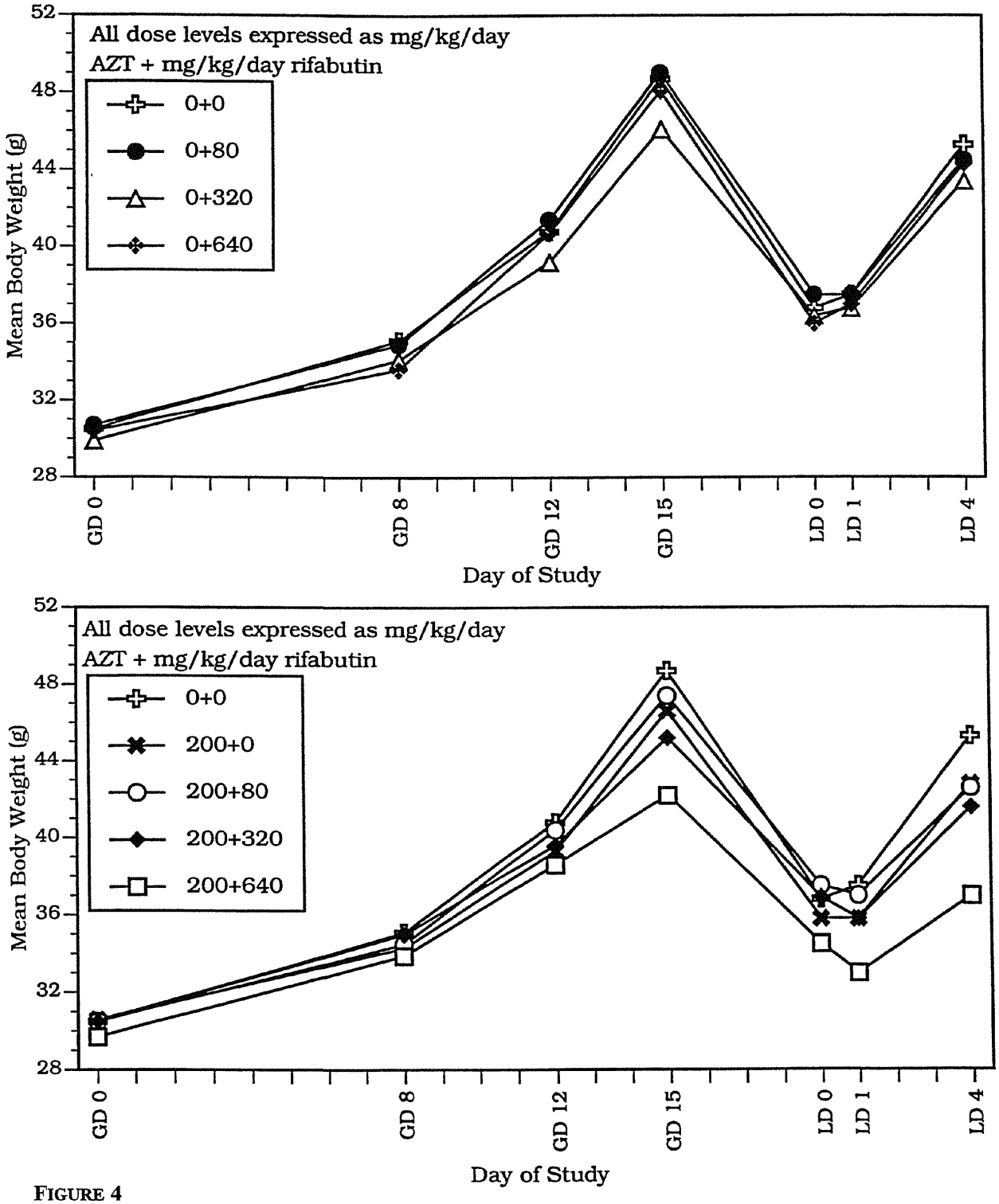


FIGURE 4

Mean Body Weights of Female-B Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations (GD=gestation day; LD=lactation day)

Note: data include only values for those dams that were actually pregnant, that survived to lactation day 4, and that delivered pups that survived to lactation day 4.

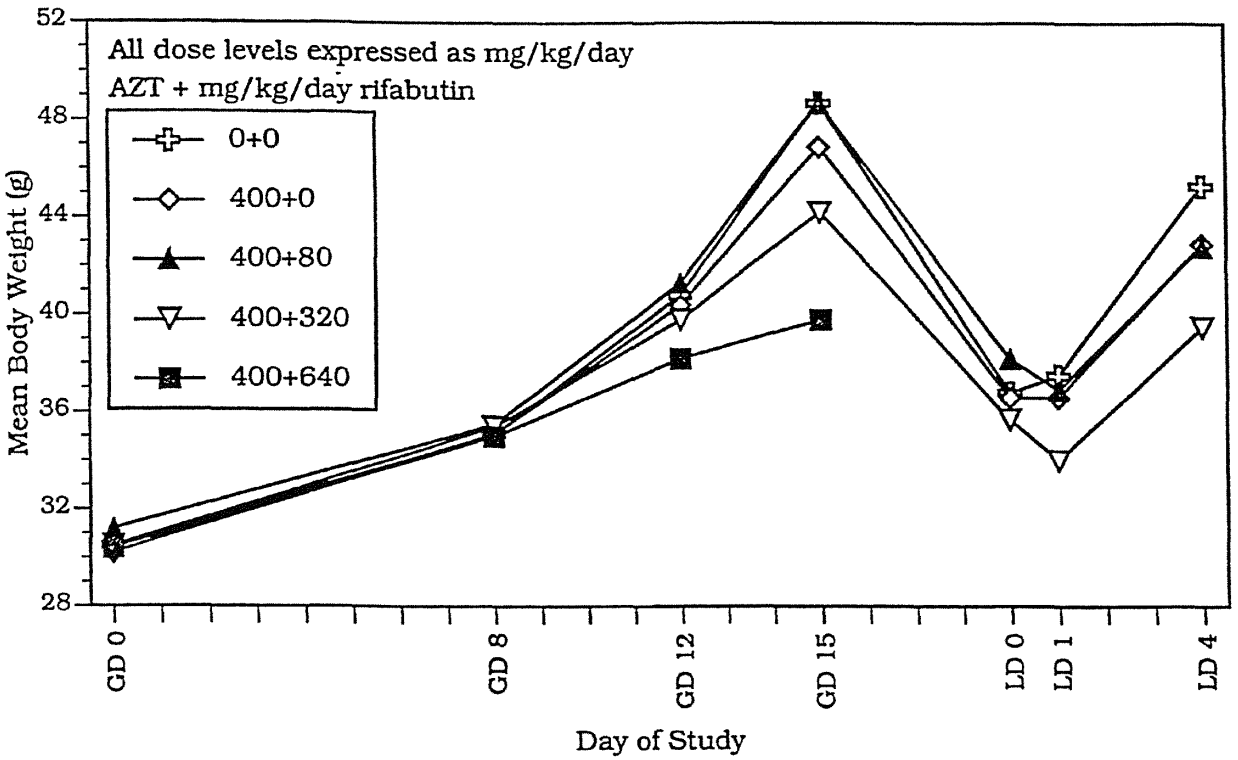


FIGURE 4

Mean Body Weights of Female-B Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations (GD=gestation day; LD=lactation day)

Note: data include only values for those dams that were actually pregnant, that survived to lactation day 4, and that delivered pups that survived to lactation day 4.

CLINICAL PATHOLOGY

Hematology

AZT Alone

Male Mice

Slight decreases in erythrocyte parameter values [erythrocyte (RBC) counts, hemoglobin concentrations (Hgb), hematocrit (Hct) values] were observed in both the 200 and 400 mg/kg AZT groups. Respective mean RBC counts (Figure 5 and Table A1) of both these groups were 12% lower ($8.36 \times 10^6/mm^3$ and $8.33 \times 10^6/mm^3$, $P \leq 0.05$) than the mean RBC count of the vehicle control group ($9.47 \times 10^6/mm^3$). Hgb concentrations and Hct values paralleled the slight decreases in RBC counts. While only statistically significant at 400 mg/kg AZT, the slight decreases in RBC counts observed with AZT administration alone were considered biologically significant due to concurrent increases in mean cell volume (MCV). Respective MCVs for male mice treated with 200 or 400 mg/kg AZT were 7% (51.6 fL) or 7% (51.7 fL) greater than the MCV of the vehicle control group (48.3 fL). While not statistically significant, these increases in MCVs were considered treatment related. These changes in erythrocyte parameters occurred with no biologically significant changes in mean reticulocyte counts.

Slightly higher mean platelet counts (Figure 6 and Table A1) were observed in both groups treated with AZT; the mean platelet count of male mice treated with 400 mg/kg AZT was 1.5 times ($1,469 \times 10^3/mm^3$) that of the mean platelet count ($992 \times 10^3/mm^3$) of the vehicle control group. Although not statistically significant, these increased platelet counts were considered biologically significant.

Administration of AZT alone was associated with lower leukocyte (WBC) counts (Figure 7 and Table A1), which could be attributed to slight decreases in both segmented neutrophil counts (Figure 8 and Table A1) and lymphocyte counts (Figure 9 and Table A1). These changes were not statistically significant but were considered biologically significant in the 400 mg/kg AZT group. Mean WBC, neutrophil, and lymphocyte counts of mice treated with 400 mg/kg AZT were, respectively, 31% ($4.81 \times 10^3/mm^3$), 32% ($0.68 \times 10^3/mm^3$), and 31% ($3.82 \times 10^3/mm^3$) lower than the respective counts in the male vehicle control group (6.97, 1.00, and $5.53 \times 10^3/mm^3$).

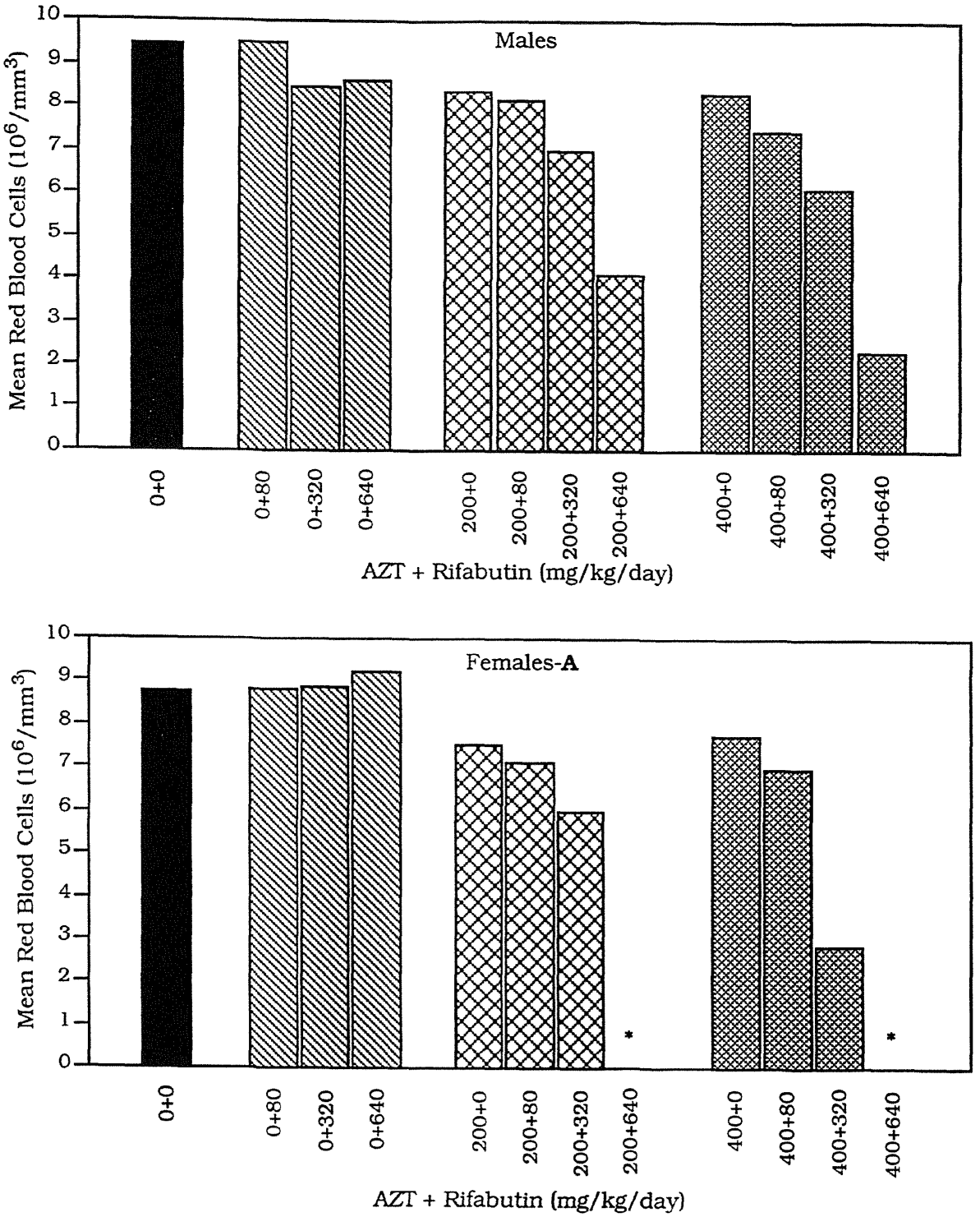


FIGURE 5

Mean Red Blood Cell Values for Male and Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations (*=data not available due to mortality. For variance and statistically significant differences, see Tables A1 and A2.)

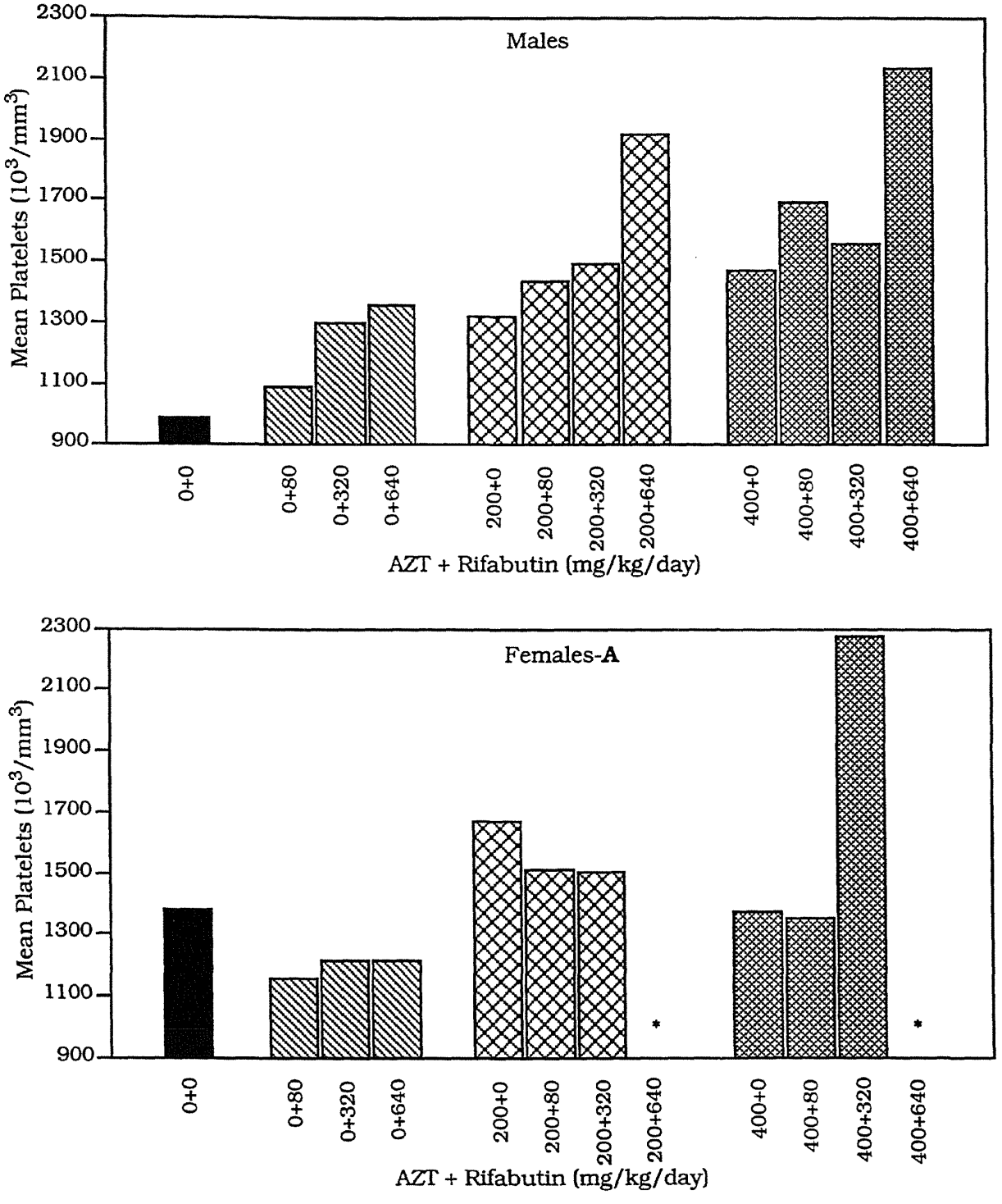


FIGURE 6
Mean Platelet Values for Male and Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations (*=data not available due to mortality. For variance and statistically significant differences, see Tables A1 and A2.)

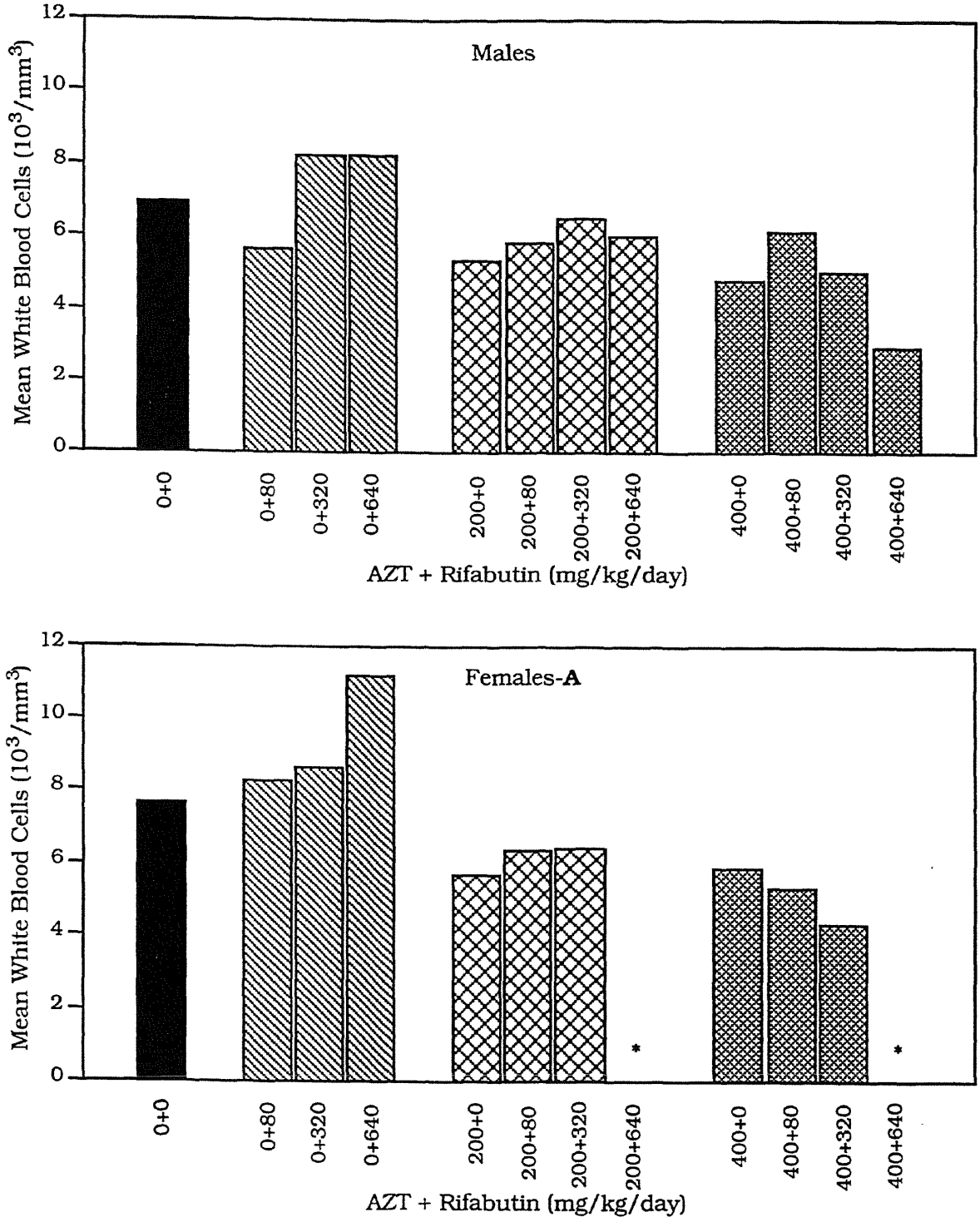


FIGURE 7
Mean Leukocyte Values for Male and Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations (*=data not available due to mortality. For variance and statistically significant differences, see Tables A1 and A2.)

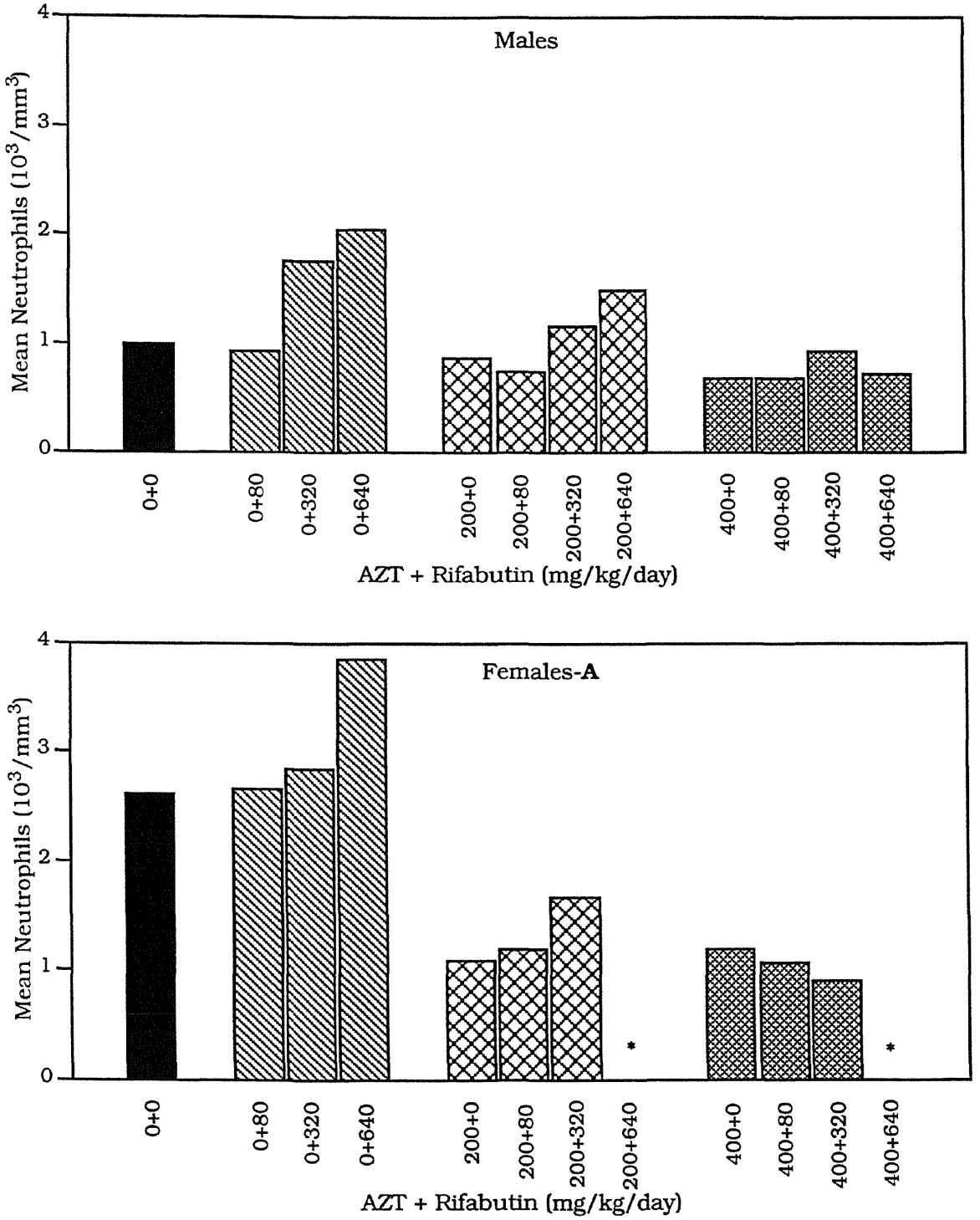


FIGURE 8

Mean Segmented Neutrophil Values for Male and Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations (*=data not available due to mortality. For variance and statistically significant differences, see Tables A1 and A2.)

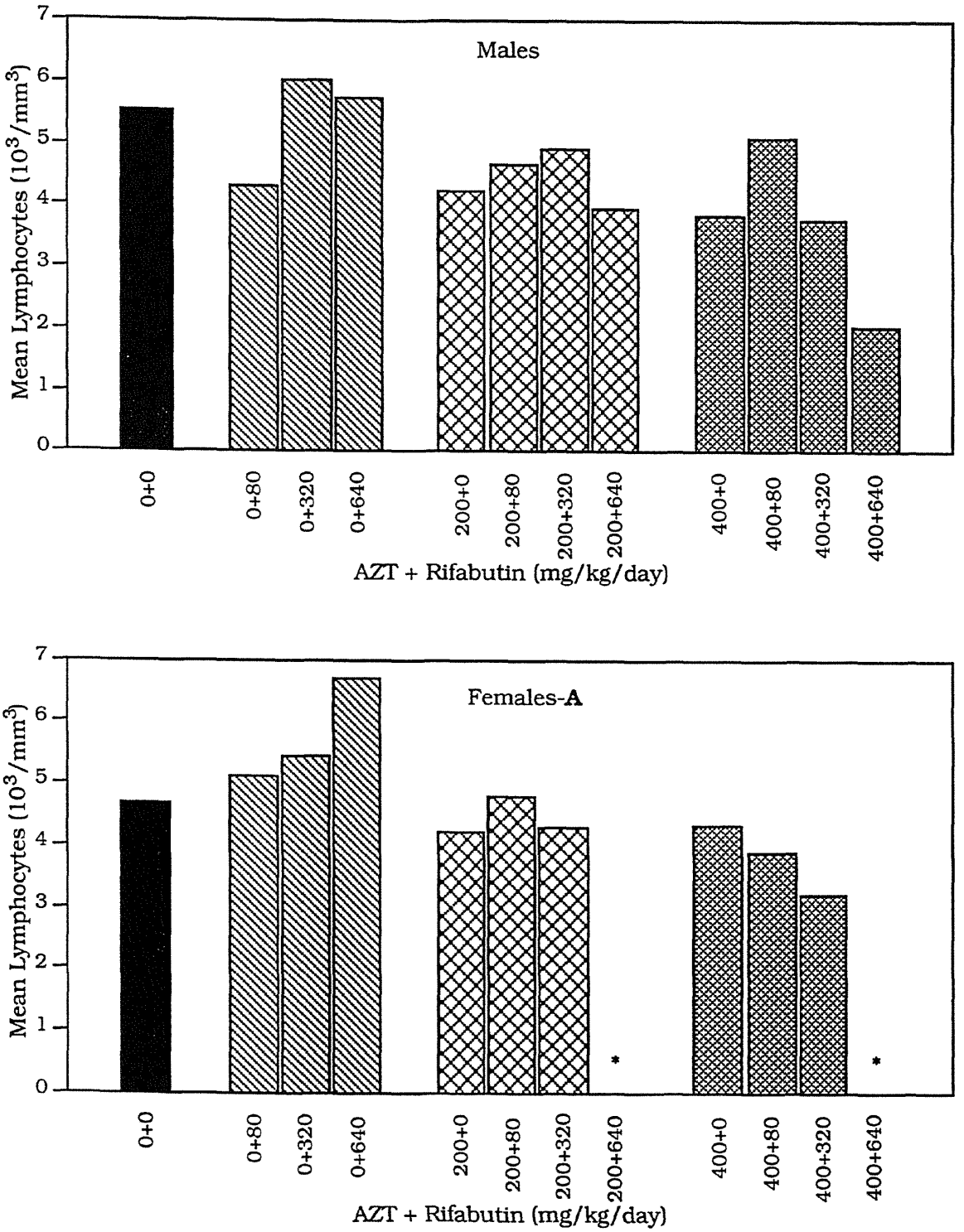


FIGURE 9

Mean Lymphocyte Values for Male and Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations (*=data not available due to mortality. For variance and statistically significant differences, see Tables A1 and A2.)

Female-A Mice

AZT administration resulted in hematologic alterations in female mice similar to those observed in male mice. RBC counts (Figure 5 and Table A2) were slightly decreased in female-A mice treated with 200 and 400 mg/kg AZT alone, with respective mean RBC counts 14% ($7.51 \times 10^6/mm^3$, $P \leq 0.01$) and 12% ($7.71 \times 10^6/mm^3$, $P \leq 0.01$) lower than the mean RBC count of the vehicle control group ($8.74 \times 10^6/mm^3$). Hgb concentrations and Hct values paralleled RBC counts. These slight but biologically significant decreases in RBC counts were accompanied by statistically significant ($P \leq 0.01$) increases in MCV. The respective MCVs of mice treated with 200 and 400 mg/kg AZT were 14% (55.9 fL) and 16% (56.8 fL) greater than the vehicle control MCV (49.1 fL). In general, alterations in mean cell hemoglobin (MCH) paralleled the changes in MCV.

An increased mean platelet count was observed only in the 200 mg/kg AZT group, although it was not statistically significant. The mean platelet count (Figure 6 and Table A2) of the female-A mice group treated with 200 mg/kg AZT was approximately 1.2 times ($1,672 \times 10^3/mm^3$) the mean platelet count ($1,382 \times 10^3/mm^3$) of the vehicle control group.

WBC counts (Figure 7 and Table A2) were slightly decreased in female-A mice treated with AZT alone. WBC counts of the 200 and 400 mg/kg AZT groups were, respectively, 26% ($5.69 \times 10^3/mm^3$, $P \leq 0.05$) and 24% ($5.86 \times 10^3/mm^3$) lower than the mean WBC count of the corresponding vehicle control group ($7.68 \times 10^3/mm^3$). These slight decreases in mean WBC counts observed in AZT-treatment groups were predominantly due to decreases in neutrophil counts (Figure 8 and Table A2). Corresponding mean neutrophil counts were respectively, 58% ($1.10 \times 10^3/mm^3$, $P \leq 0.01$) and 55% ($1.19 \times 10^3/mm^3$, $P \leq 0.01$) lower than the mean count ($2.62 \times 10^3/mm^3$) of the vehicle control group. No appreciable alterations in lymphocyte counts were observed in these groups (Figure 9 and Table A2).

Female-B Mice

Values for hematology parameters with variance and statistically significant differences are listed in Table A3. Although slight decreases in RBC counts occurred in the female-B groups administered AZT alone, these were not statistically or biologically significant. Slight increases in MCVs of mice treated with 200 and 400 mg/kg AZT of 5% (53.8 fL) and 4% (53.3 fL) above that observed in the vehicle control group (51.4 fL) suggest that altered erythropoiesis may have occurred even with only 10 days of administration of AZT. Reticulocyte counts were also slightly increased, with counts of the 200 and 400 mg/kg AZT groups 1.2 times ($5.5 \times 10^5/mm^3$) and 1.3 times ($6.1 \times 10^5/mm^3$) higher than the mean reticulocyte count of the corresponding vehicle control group ($4.6 \times 10^5/mm^3$). These data also suggest that AZT had a hematopoietic effect even with 10 days of treatment.

Platelet count was increased in only the 200 mg/kg AZT group, although the difference was not statistically significant. The mean platelet count of the 200 mg/kg AZT group was 1.2 times ($1,522 \times 10^3/mm^3$) that of the vehicle control group ($1,262 \times 10^3/mm^3$).

Rifabutin Alone

Male Mice

Administration of 320 or 640 mg/kg rifabutin alone to male mice resulted in slight decreases in RBC counts and slight increases in reticulocyte, WBC, and neutrophil counts.

Respective mean RBC counts (Figure 5 and Table A1) of male mice treated with 320 and 640 mg/kg rifabutin were approximately 11% ($8.47 \times 10^6/mm^3$) and 9% ($8.65 \times 10^6/mm^3$) lower than the mean RBC count ($9.47 \times 10^6/mm^3$) of the vehicle control group. Slight declines in Hgb concentrations ($P \leq 0.05$) and Hct values accompanied the decreases in RBC counts. While not all of the decreases in RBC parameters were statistically significant, these were considered biologically significant because they were accompanied by increases in reticulocyte counts. The MCV of the 640 mg/kg group was 5% lower (45.9 fL) than the MCV of the vehicle control group (48.3 fL), and this was considered biologically significant. Reticulocyte counts were increased in both the 320 and 640 mg/kg rifabutin groups, with the maximum increase in the 640 mg/kg group. While not statistically significant, these increases were considered treatment related. The mean reticulocyte counts of these groups were 1.5 times ($6.6 \times 10^5/mm^3$) and 1.8 times ($8.1 \times 10^5/mm^3$) the mean reticulocyte count of the vehicle control group ($4.5 \times 10^5/mm^3$).

Platelet counts (Figure 6 and Table A1) were also increased in male mice administered 320 or 640 mg/kg rifabutin, with respective mean platelet counts 1.3 times ($1,300 \times 10^3/mm^3$) and 1.4 times ($1,357 \times 10^3/mm^3$) the mean platelet count of vehicle controls ($992 \times 10^3/mm^3$).

The mean WBC counts (Figure 7 and Table A1) were slightly increased in both the 320 and 640 mg/kg rifabutin groups, with mean WBC counts 1.2 times ($8.24 \times 10^3/mm^3$, $8.26 \times 10^3/mm^3$) the mean WBC count in the vehicle control group ($6.97 \times 10^3/mm^3$). Corresponding segmented neutrophil counts (Figure 8 and Table A1) were 1.8 times ($1.75 \times 10^3/mm^3$) and 2.0 times ($2.04 \times 10^3/mm^3$, $P \leq 0.01$) the mean neutrophil count of the vehicle controls ($1.00 \times 10^3/mm^3$).

Female-A Mice

Administration of rifabutin alone to female-A mice had no impact on RBC, reticulocyte, or platelet counts. The MCV of the 640 mg/kg rifabutin group was 4% less (47.0 fL) than the mean MCV value of the vehicle control group (49.1 fL); however, this decrease in MCV was not by itself considered biologically significant.

There was a marked increase in the WBC count (Figure 7 and Table A2) of the 640 mg/kg rifabutin group. The mean WBC count of female-A mice administered 640 mg/kg rifabutin was 1.5 times ($11.18 \times 10^3/mm^3$, $P \leq 0.01$) the mean in the vehicle controls ($7.68 \times 10^3/mm^3$). Evaluation of the differential data revealed increases in both neutrophil and lymphocyte counts. The mean segmented neutrophil count (Figure 8 and Table A2) and lymphocyte count (Figure 9 and Table A2) of the 640 mg/kg group were, respectively, 1.5 times ($3.85 \times 10^3/mm^3$, $P \leq 0.05$) and 1.4 times ($6.70 \times 10^3/mm^3$) the corresponding counts of the vehicle control group. An increase in the number of large undifferentiated cells (LUC) was also observed in the 640 mg/kg group ($0.25 \times 10^3/mm^3$, $P \leq 0.01$) compared to the vehicle control group ($0.05 \times 10^3/mm^3$; Table A2). This increase in LUC may have occurred secondarily to the mild hematopoietic regenerative response with release of undifferentiated precursors or may have been associated with an atypical lymphoid or monocytoid population.

Female-B Mice

Administration of 80, 320, or 640 mg/kg rifabutin alone to female-B mice had no effect on any of the erythrocyte parameters evaluated with the exception of the reticulocyte count of the 640 mg/kg group (Table A3). The mean reticulocyte count of mice receiving 640 mg/kg rifabutin was 1.5 times ($7.0 \times 10^5/mm^3$) that observed in the vehicle control group ($4.6 \times 10^5/mm^3$). Although not statistically significant, this increase was considered biologically significant.

Platelet count also appeared to be increased in the 640 mg/kg group, with the mean platelet count for this group 1.3 times ($1,644 \times 10^3/mm^3$) that of the vehicle control group ($1,262 \times 10^3/mm^3$).

No effects on WBC counts or differentials were apparent in female-B groups treated with rifabutin alone.

AZT and Rifabutin Combinations

Male Mice

Administration of 200 or 400 mg/kg AZT + 80, 320, or 640 mg/kg rifabutin resulted in a treatment-related anemia of far greater severity than due to treatment with AZT alone. Respective mean RBC counts (Figure 5 and Table A1) of male mice treated with 200 mg/kg AZT + 80, 320, or 640 mg rifabutin were 14% ($8.15 \times 10^6/mm^3$, $P \leq 0.05$), 26% ($6.99 \times 10^6/mm^3$, $P \leq 0.01$), and 56% ($4.13 \times 10^6/mm^3$, $P \leq 0.01$) lower than the mean RBC count of the vehicle control group ($9.47 \times 10^6/mm^3$). For the groups treated with 400 mg/kg AZT + 80, 320, or 640 mg/kg rifabutin, the mean RBC counts were 22% ($7.43 \times 10^6/mm^3$, $P \leq 0.01$), 36% ($6.09 \times 10^6/mm^3$, $P \leq 0.01$), and 76% ($2.31 \times 10^6/mm^3$, $P \leq 0.01$) lower than the mean count of the vehicle control group ($9.47 \times 10^6/mm^3$). Hgb concentrations and Hct values paralleled the decreased RBC counts (Table A1).

With the exception of the 400 mg/kg AZT + 640 mg/kg rifabutin groups, macrocytic changes in erythrocytes were greater with administration of both AZT and rifabutin than with AZT alone. Increases in MCVs were apparent with 200 mg/kg AZT + 80 or 320 mg/kg rifabutin, with lesser increases apparent with 400 mg/kg AZT + 80 or 320 mg/kg rifabutin. Decreases in MCVs were apparent in the 400 mg/kg AZT + 640 mg/kg rifabutin groups. Mice treated with 200 mg/kg AZT + 80 or 320 mg/kg rifabutin had respective MCVs that were 6% (51.3 fL) and 13% (54.6 fL, $P \leq 0.01$) higher than the value of the vehicle controls (48.3 fL). The MCVs of the corresponding 400 mg/kg AZT + 80 or 320 mg/kg rifabutin groups had smaller changes in MCV with volumes 7% (51.8 fL) and 8% (52.2 fL) higher than that of the vehicle controls (48.3 fL). The high dose combination group had decreased MCVs compared to the increased MCVs observed in lower dose combination groups. The MCV for the 400 mg/kg AZT + 640 mg/kg rifabutin group was 18% lower (39.7 fL, $P \leq 0.01$) than that of the vehicle controls (48.3 fL). The marked decrease in MCVs of the 400 + 640 mg/kg group was also associated with a marked decrease in reticulocyte count, which was 76% lower ($1.1 \times 10^5/mm^3$) than the mean for the vehicle control group ($4.5 \times 10^5/mm^3$). Reticulocyte counts of other combination treatment groups tended to be increased above those observed in the vehicle controls. Counts of the 200 + 640 mg/kg group were 1.4 times ($6.3 \times 10^5/mm^3$) that of the vehicle controls ($4.5 \times 10^5/mm^3$), and counts of the 400 + 320 mg/kg group were 1.6 times ($7.3 \times 10^5/mm^3$) that of the vehicle controls ($4.5 \times 10^5/mm^3$). An additional change of a markedly increased mean cell hemoglobin concentration (MCHC) was evident in the group treated with the highest doses of both compounds, which was suggestive of hemolysis. The MCHC value was 1.5 times (40.1 g/dL, $P \leq 0.01$) the MCHC (34.9 g/dL) of the vehicle controls.

Combination administration of AZT and rifabutin caused treatment-related thrombocytosis. Respective mean platelet counts (Figure 6 and Table A1) of male mice administered 200 mg/kg AZT + 80, 320, or 640 mg/kg

rifabutin were 1.5 times (1,439 × 10^3/mm^3), 1.5 times (1,497 × 10^3/mm^3, P≤0.05), and 1.9 times (1,921 × 10^3/mm^3, P≤0.01) the mean observed in the corresponding vehicle control group (992 × 10^3/mm^3). For the groups receiving 400 mg/kg AZT + 80, 320, or 640 mg/kg rifabutin, respective mean platelet counts were 1.7 times (1,693 × 10^3/mm^3, P≤0.01), 1.6 times (1,562 × 10^3/mm^3, P≤0.05), and 2.2 times (2,135 × 10^3/mm^3, P≤0.01) times the mean platelet count of the vehicle control group (992 × 10^3/mm^3).

No biologically significant changes in differential WBC counts occurred with administration of 200 mg/kg AZT + 80, 320, or 640 mg/kg rifabutin. Treatment-related decreases in WBC, neutrophil, and lymphocyte counts were observed in male mice treated with 400 mg/kg AZT + 320 or 640 mg/kg rifabutin. Respective mean WBC counts (Figure 7 and Table A1) of male mice receiving 400 mg/kg AZT + 320 or 640 mg/kg were 28% (5.04 × 10^3/mm^3) and 58% (2.92 × 10^3/mm^3) lower than the mean of the vehicle controls (6.97 × 10^3/mm^3). Neutrophil counts (Figure 8 and Table A1) of these same two groups were 7% (0.93 × 10^3/mm^3) and 26% (0.74 × 10^3/mm^3) lower than the vehicle control group mean (1.00 × 10^3/mm^3). Lymphocyte counts (Figure 9 and Table A1) of male mice administered 400 mg/kg AZT + 320 or 640 mg/kg rifabutin were, respectively, 32% (3.77 × 10^3/mm^3) and 63% (2.05 × 10^3/mm^3) lower than the mean lymphocyte count in the vehicle controls (5.53 × 10^3/mm^3). Significant alterations were not evident in other WBC differentials.

Female-A Mice

Administration of AZT and rifabutin to female-A mice resulted in severe anemia far greater than that subsequent to the administration of AZT alone. Respective mean RBC counts (Figure 5 and Table A2) of female mice receiving 200 mg/kg AZT + 80 or 320 mg/kg rifabutin were 19% (7.11 × 10^6/mm^3, P≤0.01) and 31% (5.99 × 10^6/mm^3, P≤0.01) lower than the vehicle controls (8.74 × 10^6/mm^3). Respective mean RBC counts of female-A mice administered 400 mg/kg AZT + 80 or 320 mg/kg rifabutin were 20% (6.96 × 10^6/mm^3, P≤0.01) and 67% (2.85 × 10^6/mm^3, P≤0.01) lower than the mean of the vehicle controls (8.74 × 10^6/mm^3). Female-A mice that received 200 or 400 mg/kg AZT + 640 mg/kg rifabutin did not survive, apparently because of the severity of the anemia. In general, Hgb concentrations and Hct values decreased in a manner similar to the decreasing RBC counts. Marked increases in MCV (greater than that observed with AZT alone) were observed in female-A mice administered 200 mg/kg AZT + 80 or 320 mg/kg rifabutin and in those administered 400 mg/kg AZT + 80 mg/kg rifabutin with respective MCVs 17% (57.3 fL, P≤0.01), 18% (58.0 fL, P≤0.01), and 16% (57.1 fL, P≤0.01) higher than the MCV of the vehicle control group (49.1 fL). This macrocytosis occurred without appreciable increases in reticulocyte counts of these groups, although a slightly increased count (1.3 times the vehicle control group count) was observed in the group administered 400 mg/kg AZT + 80 mg/kg rifabutin (5.4 × 10^5/mm^3). The MCV observed in the

female-A mice administered 400 + 320 mg/kg had decreased towards a volume near normal. This group also had a marked decrease in reticulocyte count with the mean reticulocyte count 41% lower ($2.4 \times 10^5/mm^3$) than that observed in the vehicle control group ($4.1 \times 10^5/mm^3$). The MCHC of mice administered 400 + 320 mg/kg was slightly higher (36.3 g/dL) than the vehicle control group MCHC (34.1 g/dL), with two of three mice analyzed having MCHCs greater than the range observed in the vehicle control mice. In general, alterations in MCH values paralleled the changes in MCV.

Mild thrombocytosis (Figure 6 and Table A2) was present only in female-A mice administered 400 mg/kg AZT + 320 mg/kg rifabutin, with the mean platelet count 1.6 times ($2,279 \times 10^3/mm^3$) that of the vehicle control group ($1,382 \times 10^3/mm^3$).

WBC counts (Figure 7 and Table A2) were not appreciably different from the vehicle control group in female-A mice administered 200 mg/kg AZT + 80 or 320 mg/kg rifabutin. In mice administered 400 mg/kg AZT + 80 or 320 mg/kg rifabutin, WBC counts were moderately decreased, with respective mean WBC counts 31% ($5.32 \times 10^3/mm^3$, $P \leq 0.05$) and 43% ($4.34 \times 10^3/mm^3$) lower than in the vehicle controls ($7.68 \times 10^3/mm^3$). Decreases in segmented neutrophil counts (Figure 8 and Table A2) were observed in all combination treatment groups of female-A mice, with respective neutrophil counts for the groups administered 200 mg/kg AZT + 80 or 320 mg/kg rifabutin and 400 mg/kg AZT + 80 or 320 mg/kg rifabutin, 55% ($1.19 \times 10^3/mm^3$, $P \leq 0.01$), 37% ($1.66 \times 10^3/mm^3$), 59% ($1.07 \times 10^3/mm^3$, $P \leq 0.01$), and 65% ($0.91 \times 10^3/mm^3$) lower than the mean count of the vehicle control group ($2.62 \times 10^3/mm^3$). Biologically significant differences in other WBC differentials were not observed (Table A2).

Female-B Mice

Values for hematology parameters with variance and statistically significant differences are listed in Table A3. Slight decreases in RBC count were observed only in female-B mice administered 200 or 400 mg/kg AZT + 640 mg/kg rifabutin, with the respective mean RBC counts of these groups 10% ($7.63 \times 10^6/mm^3$, $P \leq 0.05$) and 13% ($7.33 \times 10^6/mm^3$, $P \leq 0.01$) lower than that of the corresponding vehicle controls ($8.44 \times 10^6/mm^3$). While the other combination treatment groups of female-B mice did not exhibit significant changes in mean RBC counts, low individual RBC counts were present in all combination treatment groups, with the exception of 200 + 80 mg/kg, when counts were compared to the range of counts observed in the vehicle control group. In general, Hgb concentrations and Hct values paralleled RBC counts. In addition to low incidences of anemia in all combination treatment groups, MCVs were increased in all combination treatment groups in an apparent dose-related manner. The MCVs of female-B mice treated with 200 mg/kg AZT + 80, 320, or 640 mg/kg rifabutin were, respectively, 5% (54.0 fL), 6% (54.7 fL, $P \leq 0.01$), and 10%

(56.6 fL, $P \leq 0.01$) higher than the vehicle control MCV (51.4 fL). The MCVs of mice treated with 400 mg/kg AZT + 80, 320, or 640 mg/kg rifabutin were 4% (53.4 fL), 6% (54.4 fL, $P \leq 0.05$), and 12% (57.4 fL, $P \leq 0.01$) higher than that of the vehicle control group (51.4 fL). In general, a slight increase in reticulocyte numbers paralleled the increase in MCV. In female-B mice administered 200 mg/kg AZT + 320 or 640 mg/kg rifabutin, mean reticulocyte counts were 1.2 times ($5.7 \times 10^5/mm^3$) and 1.6 times ($7.3 \times 10^5/mm^3$) that of the vehicle controls ($4.6 \times 10^5/mm^3$). In mice administered 400 mg/kg AZT + 80, 320, or 640 mg/kg rifabutin, mean reticulocyte counts were, respectively, 1.2 times ($5.5 \times 10^5/mm^3$), 1.6 times ($7.4 \times 10^5/mm^3$), and 2.0 times ($9.0 \times 10^5/mm^3$) that of the vehicle controls ($4.6 \times 10^5/mm^3$). There were marginal changes in MCHC in some of the combination treatment groups, and they were not considered biologically significant.

Platelet counts were appreciably increased only in combination treatment groups treated with 200 or 400 mg/kg AZT + 640 mg/kg rifabutin, with respective mean platelet counts 1.3 times ($1,608 \times 10^3/mm^3$) and 1.4 times ($1,786 \times 10^3/mm^3$) the vehicle controls ($1,262 \times 10^3/mm^3$).

Increases in mean WBC count were observed in female-B mice administered 200 + 640 mg/kg and in groups administered 400 mg/kg AZT + 320 or 640 mg/kg rifabutin, and were 1.3 times ($6.40 \times 10^3/mm^3$), 1.4 times ($6.71 \times 10^3/mm^3$), and 1.7 times ($8.46 \times 10^3/mm^3$) higher than counts of the vehicle control group ($4.92 \times 10^3/mm^3$). Slight increases in segmented neutrophil count and lymphocyte count accompanied increases in WBC count, with the maximum change observed in the 400 + 640 mg/kg group. Mean neutrophil and lymphocyte counts in this group were 2.1 times ($2.27 \times 10^3/mm^3$) and 1.6 times ($5.63 \times 10^3/mm^3$) higher than the corresponding counts observed in vehicle control group ($1.06 \times 10^3/mm^3$, $3.54 \times 10^3/mm^3$). Other differential counts were either unchanged (basophils, LUC) or slightly increased (monocytes, eosinophils).

Hematology Summary

Treatment with AZT alone resulted in mild anemia in both male and female mice with macrocytic changes of the erythrocytes and no appreciable changes in reticulocyte numbers. The changes in erythrocyte parameters were most prominent in the female-A mice and less prominent in the males with few changes in the female-B mice. This pattern of response parallels the duration of treatment with the female-A mice having received AZT for the longest period (28 to 32 days) of time and the female-B mice having received AZT for the shortest period (10 days). The greater effect of prolonged treatment on RBC counts is due to both the short life span (approximately 30 days) of erythrocytes in mice (Jain, 1986) and possibly also due to a cumulative effect of AZT. Macrocytosis, as observed in this study, is a common observation in humans treated with AZT (Snower and Weil, 1993). The etiology for this macrocytosis is uncertain, but AZT may cause a drug-induced macrocytosis by inhibition of thymidylate kinase (via AZT monophosphate) with decreased levels of thymidine

triphosphate available for DNA synthesis, or by inhibition of mammalian DNA polymerase which occurs at a 100-fold greater concentration of AZT than that necessary to inhibit reverse transcriptase (Herzlich *et al.*, 1990; Snower and Weil, 1993). In male mice, the mild anemia observed with AZT was accompanied by histopathologic observations of splenic hematopoietic cell proliferation in both the 200 and 400 mg/kg AZT treatment groups. This indicates a mild regenerative stimulus is present, even though reticulocytosis was not evident.

The sporadic mild thrombocytosis observed in all three groups is probably due to the overall mild regenerative response present and probably indicates a lesser effect of AZT alone on platelet production than on RBC production. The decreases in WBC, neutrophil, and lymphocyte counts observed in males and female-A mice and the lack of such an effect in the female-B mice suggest a mild duration of treatment-related cumulative effect of AZT on production of neutrophils and lymphocytes.

In evaluating the results from all three groups of mice treated with rifabutin alone, rifabutin was associated with slightly decreasing RBC counts (male mice only) and increasing reticulocyte counts (male and female-B mice), and decreasing mean cell volumes (male and female-A mice) at the high doses of rifabutin only. These results suggest that rifabutin alone causes a mild normocytic regenerative anemia at lower doses; however, at 640 mg/kg, either peripheral destruction of erythrocytes or altered hematopoiesis leads to production of smaller erythrocytes. The sporadic increases in platelet counts observed in male and female-B mice administered rifabutin alone are compatible with an overall nonspecific response to a regenerative stimulus. Splenic hematopoietic cell hyperplasia was observed in all three rifabutin groups in male mice, indicating that a regenerative stimulus was present.

The mild leukocytosis and neutrophilia observed in male and female-B mice groups administered rifabutin alone may have been due to the overall regenerative stimulus; however, they may also have been due to an inflammatory stimulus. Bone marrow myeloid hyperplasia was observed in a small number of male mice and is consistent with lung and sporadic liver mixed cell infiltrates observed in male mice in the 320 and 640 mg/kg rifabutin groups. These inflammatory foci may have been associated with the leukocyte responses. In female-A mice administered rifabutin alone, mixed cell infiltrates in the liver were also present in a majority of the 640 mg/kg group, and some of the mice in the 320 mg/kg rifabutin group. Additionally, chronic active inflammation observed histopathologically in the stomach in 11 of 17 female mice in the 640 mg/kg group may also have contributed to an inflammatory response.

The hematologic changes that occurred with the combination of AZT and rifabutin indicated a more severe anemia with the combination therapy than with either compound alone. The hematopoietic toxicity observed

with combination therapy was both dose- and duration-of-treatment-dependent. The female-A mice, which were administered AZT and rifabutin for the longest period (28 to 32 days), exhibited the most severe anemia. The female-B mice, which were administered the combination of AZT and rifabutin for only 10 days, exhibited the smallest changes in erythrocyte counts. The MCV increased early in lower dose combination groups and decreased late in higher dose combination groups. These changes indicate that dysplastic erythropoiesis was occurring, with a regenerative stimulus present at early time points and at low dose combinations, but not at high dose combination treatments. This and the decrease in reticulocyte count observed with time and increased dose suggest a cumulative effect of the combination of AZT and rifabutin. At the higher dose combinations, the microcytic erythrocytic changes observed could also indicate a hemolytic component, which is also suggested by the increased MCHCs; however, the primary component was considered to be erythroid hypoplasia. Splenic hematopoietic cell proliferation and erythroid depletion of the bone marrow was observed in male mice administered 200 or 400 mg/kg AZT + 320 or 640 mg/kg rifabutin, further indicating more severe hematopoietic toxicity.

The mild leukopenia and decreases in neutrophil and lymphocyte counts observed in male mice treated with AZT and rifabutin occurred in spite of the presence of mixed cell infiltrates in the lung, liver, and stomach observed histologically. This suggests that the mice were not able to mount an appropriate response to the inflammatory stimulus due to hematopoietic cell damage. In female-A mice, mixed cell infiltrates were also prominent in the liver and stomach of some of the combination therapy groups, concurrent with leukopenia.

Clinical Chemistry

AZT Alone

Values for clinical chemistry parameters with variance and statistically significant differences are listed in Tables A4 and A5. Evidence of liver toxicity was not observed with administration of 200 or 400 mg/kg AZT in male mice. Slight nonspecific increases in mean alkaline phosphatase (ALP) activities were observed in female-A mice, with mean ALP activities of 200 and 400 mg/kg AZT groups both 1.4 times (71 IU/L, 74 IU/L) the ALP activity of the vehicle control group (52 IU/L). This was not considered to be treatment related due to the lack of a dose-related effect and the lack of consistent corresponding hepatic lesions in these mice.

Rifabutin Alone

No evidence of liver toxicity was observed with 80 mg/kg rifabutin in either male or female mice. Treatment-related increases in mean alanine aminotransferase (ALT) activity were observed in male and female-A mice treated with 320 or 640 mg/kg rifabutin. The mean ALT activities of male mice in the 320 and 640 mg/kg rifabutin groups were 1.3 times (35 IU/L) and 2.5 times (65 IU/L) the mean ALT activity of the corresponding vehicle control group (26 IU/L). In female-A mice, the ALT activities for the 320 and 640 mg/kg rifabutin groups were 1.9 times (75 IU/L, $P \leq 0.05$) and 4.1 times (159 IU/L, $P \leq 0.01$) the mean ALT activity of the vehicle control group (39 IU/L). Accompanying these changes in female-A mice were increases in mean aspartate aminotransferase (AST) activity and mean bile acid concentration (Figure 10 and Table A5) of the 640 mg/kg rifabutin group, with values 1.7 (200 IU/L) and 2.1 (31 μM/L, $P \leq 0.01$) times the corresponding vehicle control values (118 IU/L, 15 μM/L).

In mice administered rifabutin alone, changes in ALT and AST activities and mean bile acid concentrations were considered to be related to the histopathologic changes of mixed cell inflammation and possibly focal necrosis in the liver, even though not all mice with histopathologic changes had alterations in clinical chemistry parameters. The slight decreases in Hct concentration observed in mice administered rifabutin alone were not sufficient to lead to tissue anoxia and contribute to the changes in liver enzyme activity or bile acid concentrations.

AZT and Rifabutin Combinations

No evidence of liver toxicity was observed in the 200 + 80 mg/kg groups of male and female-A mice. Male mice in the 400 + 80 mg/kg group had a mean sorbitol dehydrogenase (SDH) activity 1.4 times the mean SDH activity of the vehicle control group; however, this was not considered biologically significant as only one of nine mice had increased SDH values as compared to the range observed in vehicle controls. Female mice in the 400 + 80 mg/kg group had no changes in clinical chemistry parameters suggestive of liver toxicity.

Male mice receiving 200 or 400 mg/kg AZT + 320 mg/kg rifabutin had mean ALT activities 1.4 times (36 IU/L) and 1.6 times (41 IU/L) the vehicle control group (26 IU/L). SDH activity was also increased in the 400 + 320 mg/kg group, with mean SDH activity 1.5 times (30 IU/L) the mean SDH activity observed in vehicle controls (20 IU/L). In female-A mice in the 200 + 320 mg/kg group, the mean ALT activity was 2.5 times (97 IU/L, $P \leq 0.01$) that of vehicle control group (39 IU/L), and the total bile acid concentration (Figure 10) was 1.7 times (25 μM/L) that of the vehicle control group (15 μM/L). Two of three female mice in the 400 + 320 mg/kg group, which had survived to hematology sampling, had increased ALP activities and total bile acid concentrations (Figure 10 and Table A5) when compared to the ranges in the vehicle control

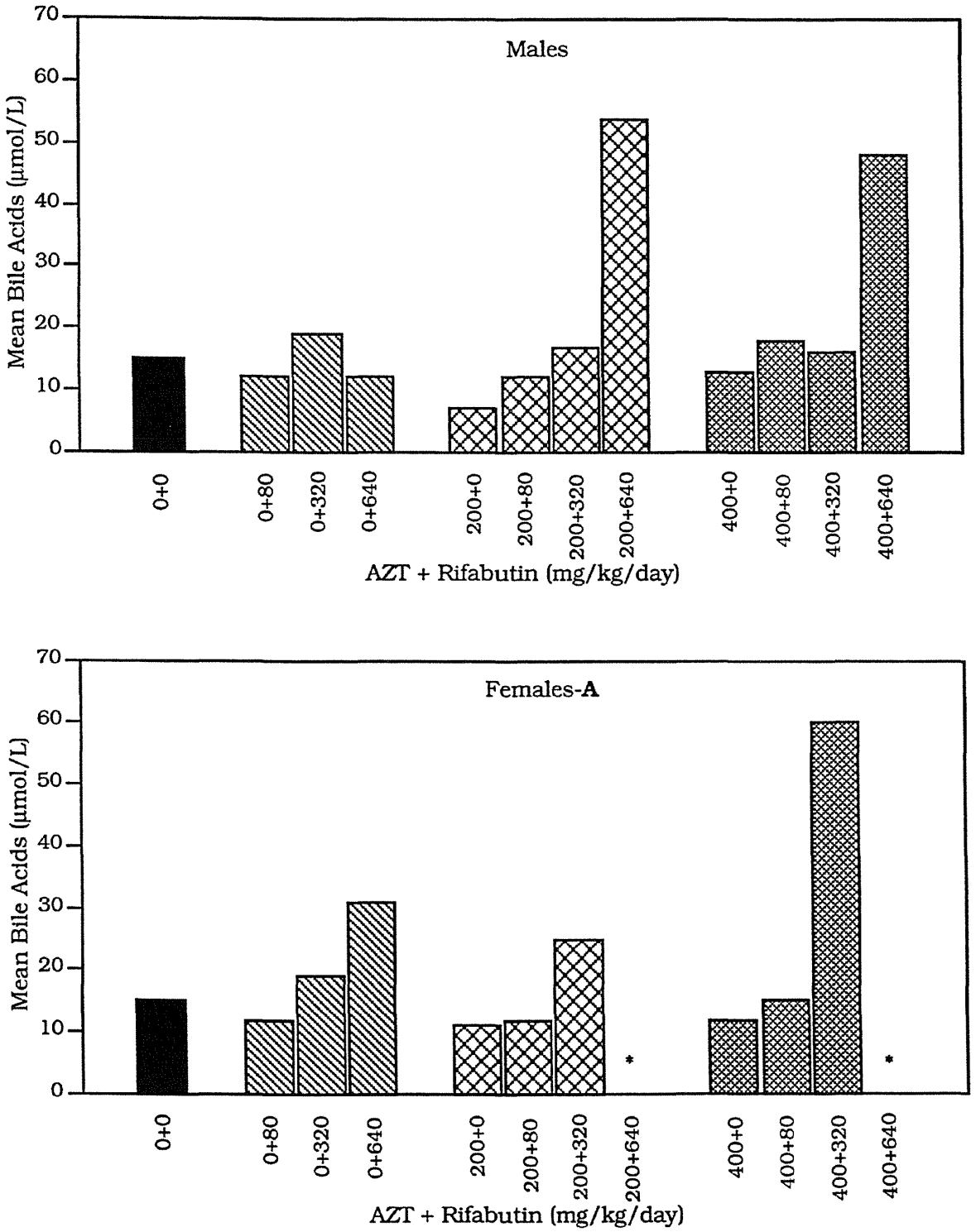


FIGURE 10
Mean Bile Acid Values for Male and Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations (*=data not available due to mortality. For variance and statistically significant differences, see Tables A4 and A5.)

group. Mean values for ALP activity and total bile acid concentration in this group were 1.6 times (82 IU/L) and 4.0 times (60 μM/L, $P \leq 0.01$) that of the vehicle control group (52 IU/L, 15 μM/L).

Male mice in the 200 or 400 mg/kg AZT + 640 mg/kg rifabutin groups had increases in total bile acid concentration (Figure 10 and Table A4). Mean total bile acid concentrations of these two groups were 3.6 times (54 μM/L, $P \leq 0.01$) and 3.2 times (48 μM/L, $P \leq 0.05$) that of the vehicle control group (15 μM/L). Data were not available from female-A mice treated with AZT + 640 mg/kg rifabutin because of early mortality.

Administration of 200 or 400 mg/kg AZT + 80 mg/kg rifabutin was not associated with any significant changes in liver enzyme activity or bile acid concentration. Administration of 200 mg/kg AZT + 320 or 640 mg/kg rifabutin was associated with similar or lower increases in ALT activity than observed with rifabutin alone. The reason for this is not clear. Male mice, but not female mice, had increases in SDH activities with combination treatment. Consistent increases in bile acid concentration were observed in males treated with 200 or 400 mg/kg AZT + 640 mg/kg rifabutin and in female mice treated with 200 or 400 mg/kg AZT + 320 mg/kg rifabutin. The increases in liver enzyme activity were consistent with histopathologically observed liver pathology of mixed cell infiltration and possibly focal necrosis. The increases in mean bile acid concentration in AZT/rifabutin groups may have been due to greater incidences of severe anemia in these groups. Severe anemia could have led to tissue anoxia, which would correspond with the centrilobular atrophy observed histopathologically in the higher dose combination treatment groups. Since an increase in mean bile acid concentration was also observed with administration of rifabutin alone in females in the 640 mg/kg rifabutin group, and other enzyme activity values were affected, the anemia does not account for all of the changes in hepatic enzyme and bile acid activities observed in AZT/rifabutin combination treatment or rifabutin alone.

Plasma AZT, GAZT, and Rifabutin Concentrations

Plasma concentrations of AZT, AZT-β-D-glucuronide (GAZT), and rifabutin in male and female-A mice determined at 15 to 90 minutes after the last dose are listed in Table 4. In male mice that received AZT at 400 mg/kg with rifabutin at 320 or 640 mg/kg, plasma concentrations of AZT and GAZT appeared to have increased when coadministered with 640 mg/kg of rifabutin as compared to the concentrations when coadministered with 320 mg/kg of rifabutin (Figure 11). In male mice that received rifabutin at 320 mg/kg, the plasma concentrations of rifabutin appeared to be similar with or without AZT coadministration. However, at 640 mg/kg of rifabutin, the plasma concentrations of rifabutin appeared to have increased when coadministered with AZT (Figure 13). Thus, in male mice there appear to be an interaction between AZT at

400 mg/kg and rifabutin at 640 mg/kg causing increases in plasma concentrations of both compounds. In female-A mice coadministration of 400 mg/kg of AZT with 80 and 320 mg/kg of rifabutin did not appear to affect the plasma concentrations of AZT, GAZT (Figure 12) or rifabutin (Figure 14). Results on the effect of coadministration of AZT at 400 mg/kg with rifabutin at 640 mg/kg are not available due to early mortality of female-A mice.

TABLE 4
Mean Concentrations of AZT, GAZT, and Rifabutin (μg/mL) in Plasma for Male and Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations

	AZT Sample Time (minutes)				GAZT Sample Time (minutes)				Rifabutin Sample Time (minutes)			
Dose[a]	15	30	60	90	15	30	60	90	15	30	60	90
Male												
0 + 320	0.153	ND[b]	0.089	ND	ND	ND	ND	ND	11.1	12.8	15.9	15.7
0 + 640	0.121[c]	ND	0.102[c]	ND	ND	ND	ND	ND	9.5	13.8	15.2	26.5
400 + 320	71.8	68.7	38.1	14.2	1.03	1.63	0.740	0.161	10.9	12.1	19.8	14.1
400 + 640	91.5	77.1	62.5	27.9	1.55	1.62	1.29	0.398	15.3	27.6	29.5	24.0
Female-A												
0 + 80	0.088	ND	0.994[c]	ND	ND	ND	ND	ND	4.57	5.06	5.62	5.65
0 + 320	ND	0.204	0.817[c]	ND	ND	ND	ND	ND	10.2	11.0	14.6	20.8
0 + 640	0.152	0.152[c]	0.696	0.155[c]	ND	ND	ND	ND	9.67	12.3	18.7	14.5
400 + 80	101.7	74.9	86.4	37.4	0.598	0.719	1.27	0.273	4.56	5.31	8.01	7.37
400 + 320	83.5	82.3	78.8	52.4	0.587	0.766	1.24	0.462	8.57	11.8	15.7	20.1

[a] Daily gavage doses of AZT + rifabutin (mg/kg per day)
[b] ND=Not detected; estimated detection limit for AZT=0.042; estimated detection limit for GAZT=0.078
[c] AZT concentration is based upon the value for one animal/time point; AZT was not detected in the sample from the other animal at the same time point.

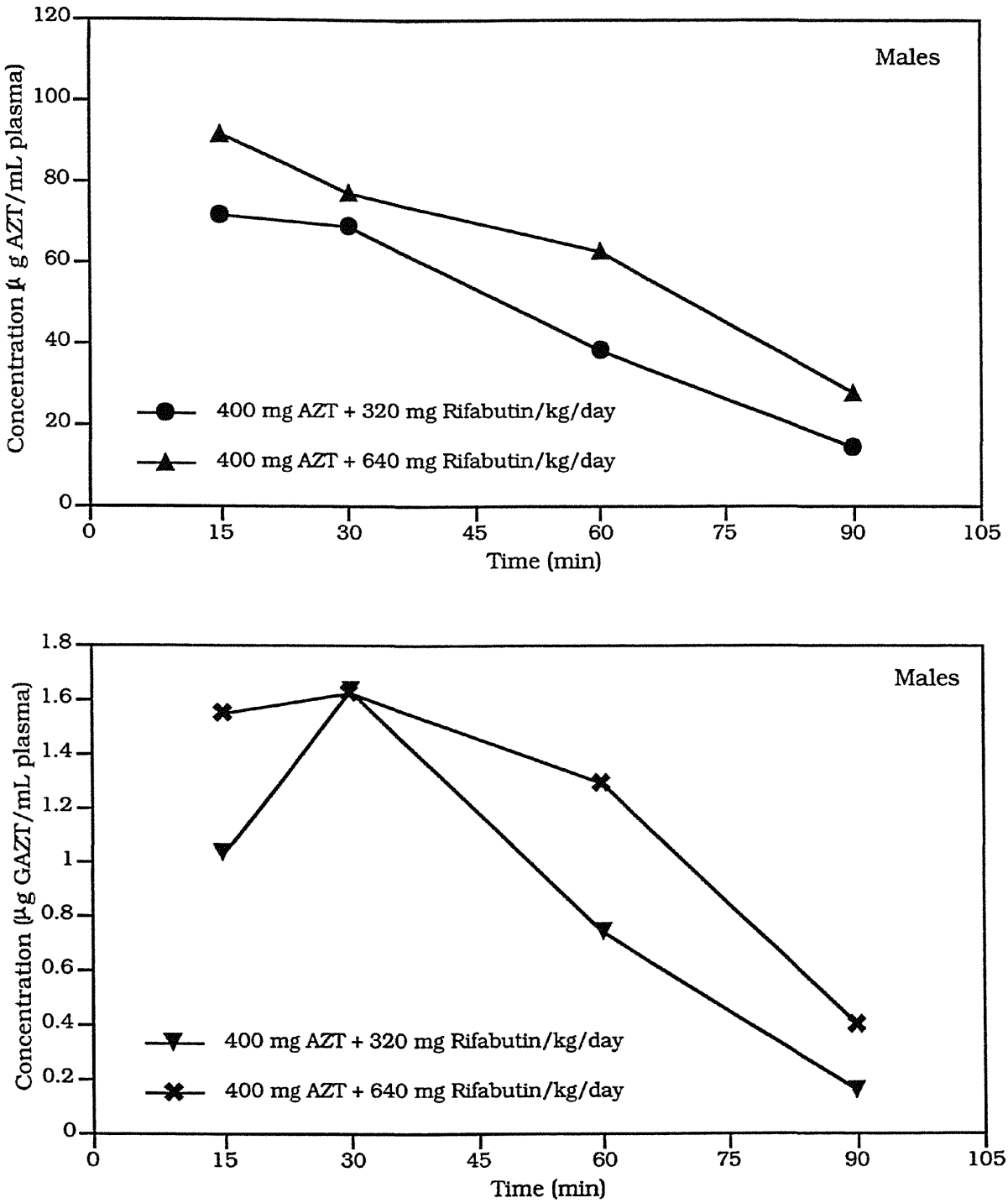


FIGURE 11
Plasma Concentrations of AZT and GAZT for Male Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations

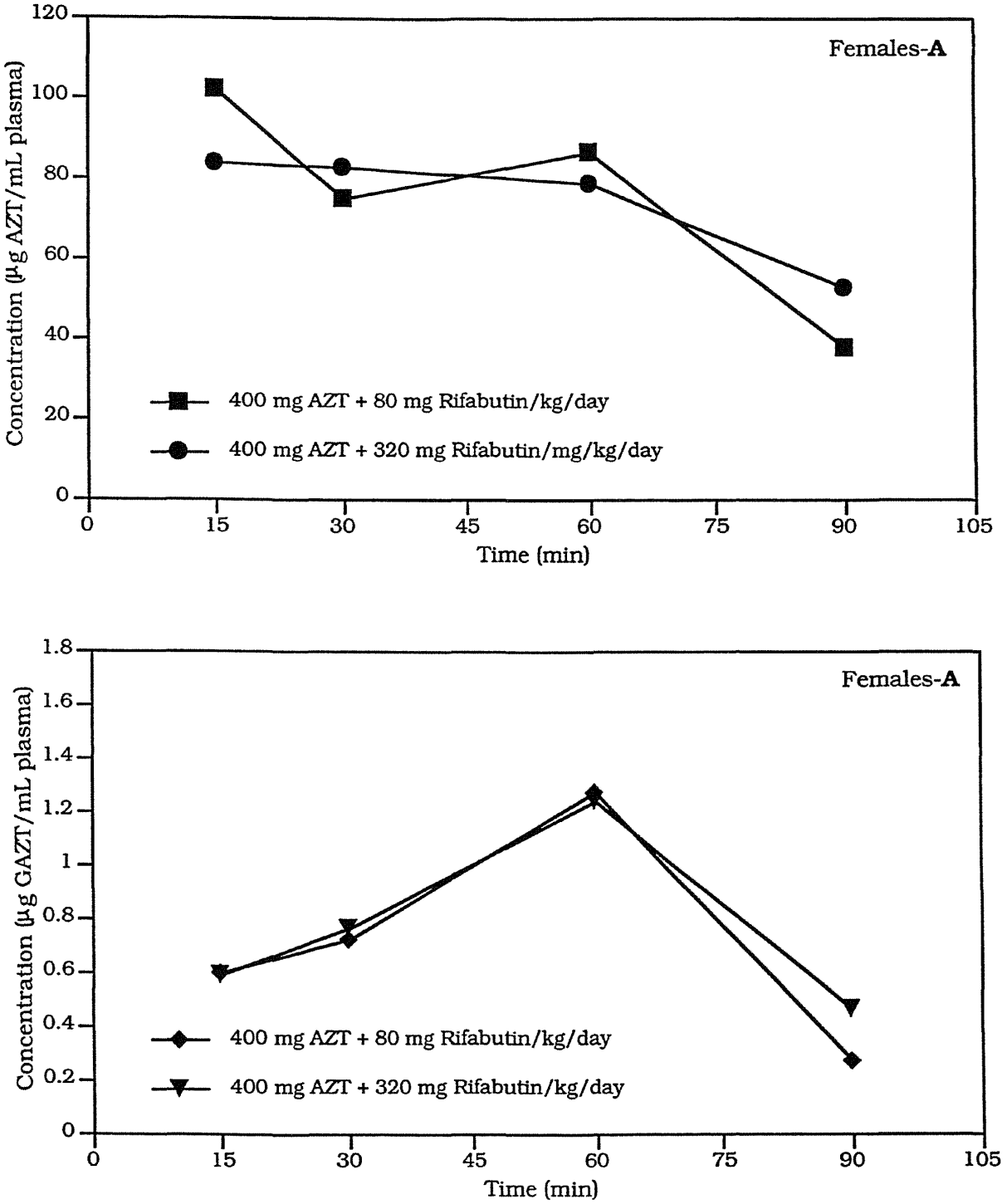


FIGURE 12
Plasma Concentrations of AZT and GAZT for Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations

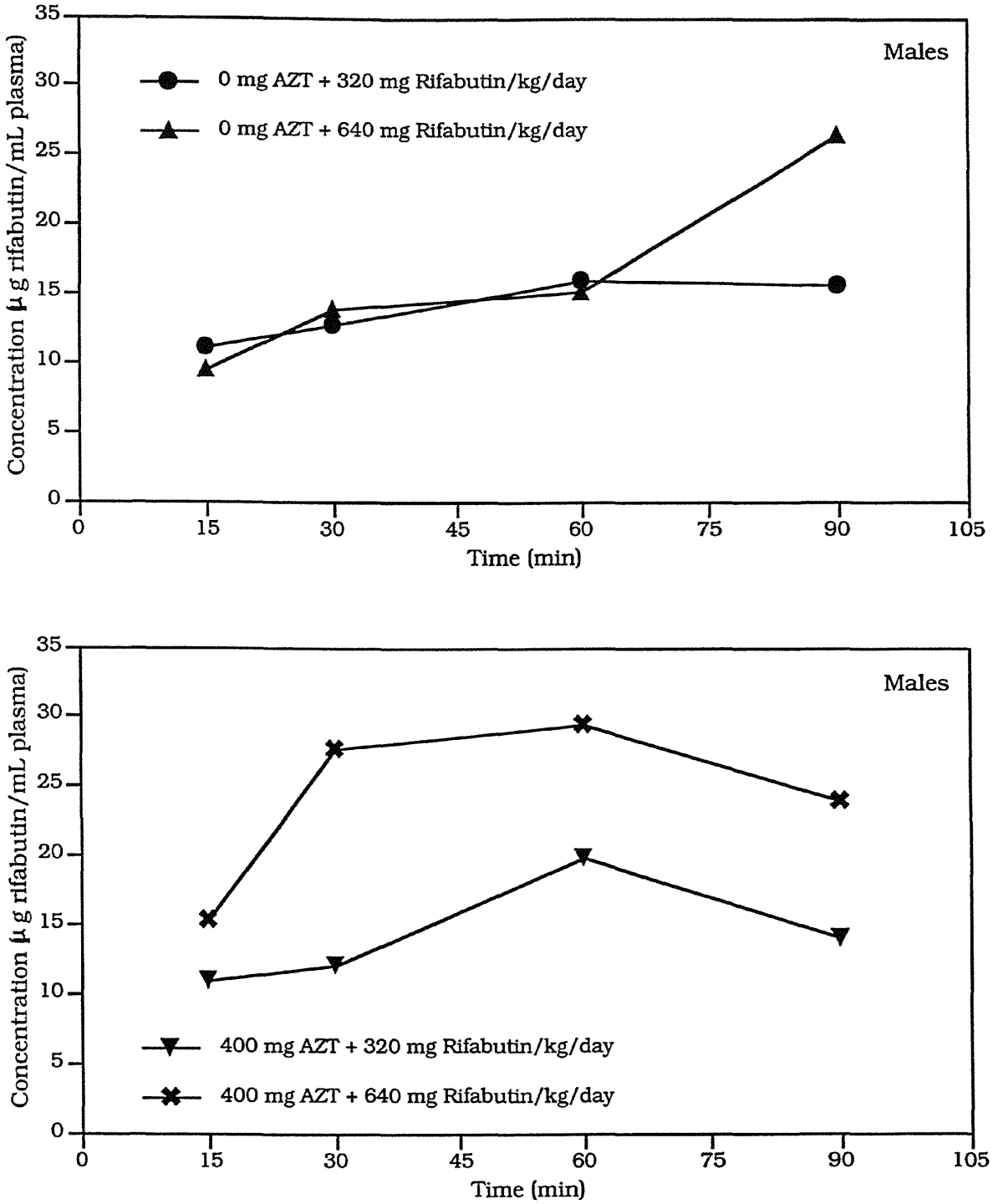


FIGURE 13
Plasma Concentrations of Rifabutin for Male Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations

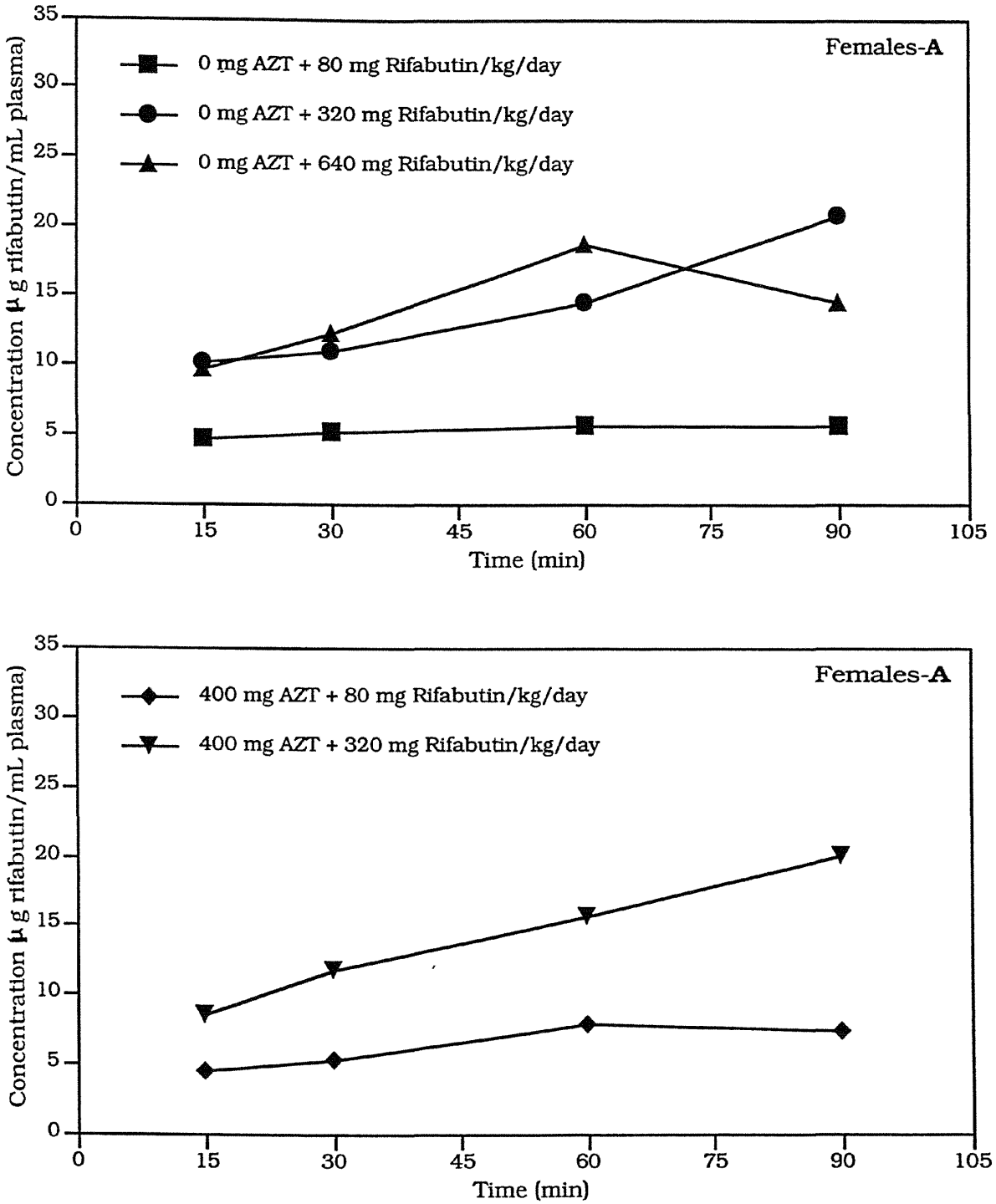


FIGURE 14
Plasma Concentrations of Rifabutin for Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations

NECROPSY OBSERVATIONS

Gross lesions attributed to treatment with AZT, rifabutin, and AZT + rifabutin were seen in male, female-A, and female-B mice. Treatment-related lesions in male mice consisted of red carcass, small thymus, small spleen, enlarged spleen, mottled stomach, and foci in the stomach. Treatment-related lesions in female-A mice consisted of red carcass, small thymus, small spleen, enlarged spleen, mottled stomach, foci in the stomach, thick stomach, red stomach, and dilatation of the gallbladder. Red carcass was found only in male and female-A mice treated with rifabutin alone or AZT + rifabutin. Treatment-related lesions in female-B mice consisted of small thymus, enlarged spleen, and thick stomach. Nodular bone lesions, which involved the ventral aspect of the thoracic vertebrae or ribs near their junction with the vertebrae, were observed in most male and female-A mice and a few female-B mice. This lesion was attributed to mechanical irritation caused by the gavage needle during dosing. The remaining gross lesions were considered to be incidental or spontaneous.

HISTOPATHOLOGIC OBSERVATIONS

Photomicrographs of representative treatment-related lesions in adult mice (with accompanying summary, Table 5) are shown in Plates 1 through 17.

TABLE 5
Summary of Microscopic Examinations of Compound-Related Lesions in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations

Dose and Treatment Group[a]	Tissue	Magnification	Plate Number	Treatment-Related Lesions
0 + 0, male	Liver	160×	1	None
400 + 640, male	Liver	160×	2	Hepatocellular cytoplasmic alteration, centrilobular atrophy
0 + 640, female-A	Liver	400×	3	Mixed cellular infiltration
0 + 0, male	Spleen	160×	4	None
400 + 640, male	Spleen	160×	5	Hematopoietic cell proliferation
400 + 640, male	Spleen	160×	6	Atrophy of red pulp and lymphoid follicles
0 + 0, male	Thymus	160×	7	None
400 + 640, male	Thymus	160×	8	Thymic atrophy

TABLE 5
Summary of Microscopic Examinations of Compound-Related Lesions in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations

Dose and Treatment Group	Tissue	Magnification	Plate Number	Treatment-Related Lesions
0 + 0, male	Mesenteric lymph node	160×	9	None
400 + 640, male	Mesenteric lymph node	160×	10	Atrophy of lymphoid cells
0 + 0, male	Bone marrow	160×	11	None
0 + 640, male	Bone marrow	160×	12	Myeloid cell hyperplasia
400 + 640, male	Bone marrow	160×	13	Depletion of erythroid cells
0 + 0, male	Glandular stomach	160×	14	None
0 + 640, male	Glandular stomach	160×	15	Cystic degeneration
0 + 0, male	Forestomach	160×	16	None
400 + 640, male	Forestomach	160×	17	Epithelial hyperplasia, chronic active inflammation

[a] Daily gavage doses of AZT + rifabutin (mg/kg per day)

Treatment-Related Lesions

AZT

Treatment-related lesions were seen in the liver of male and female-A mice and in the spleen of male mice. The treatment-related liver change (Tables 6 and 7) observed in male and female mice was designated "cytoplasmic alteration, hepatocyte." This consisted of a homogeneous, eosinophilic appearance of the hepatocellular cytoplasm and was suggestive of the absence of normally occurring glycogen. This morphologic alteration evident in hepatocytes likely represents a physiological response to diminished food intake (Harada *et al.*, 1999) and weight loss as opposed to a direct cellular response to the test article. The change was present primarily in the centrilobular areas. Hepatocellular cytoplasmic alteration was graded for severity based on the following criteria:

Minimal - approximately 15% or less of hepatocytes with cytoplasmic alteration

Mild - approximately 16% to 50% of hepatocytes with cytoplasmic alteration

Moderate - approximately 51% to 90% of hepatocytes with cytoplasmic alteration

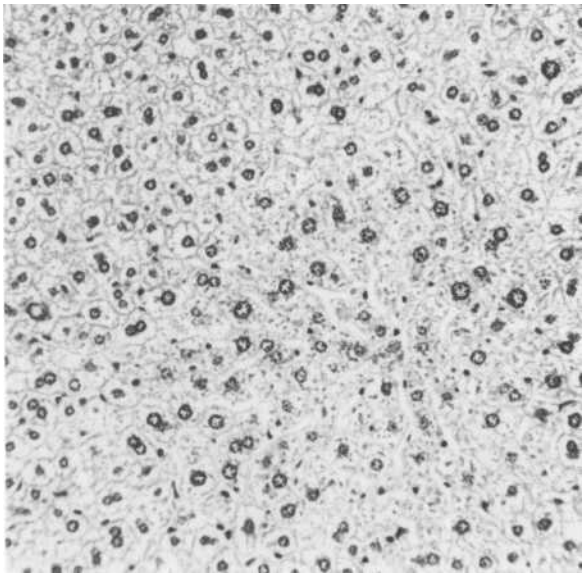


PLATE 1
Liver of a vehicle control male Swiss (CD-1®) mouse showing no lesions. H&E; 160 x

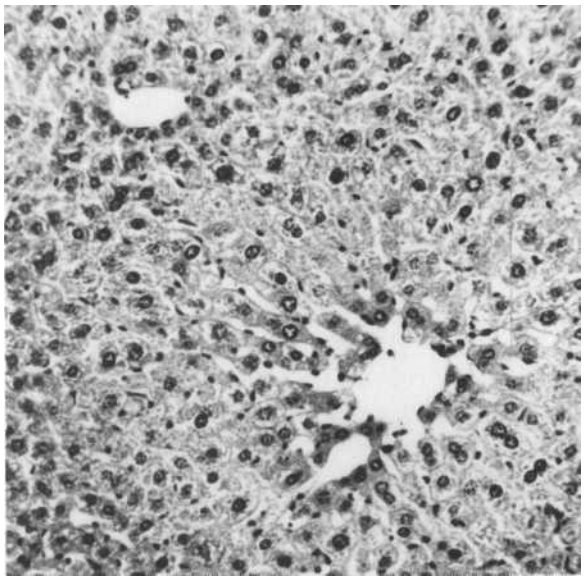


PLATE 2
Liver of a male Swiss (CD-1®) mouse given 400 mg AZT + 640 mg rifabutin per kg body weight per day by gavage for 3 weeks showing hepatocellular cytoplasmic alteration and centrilobular atrophy. H&E; 160x

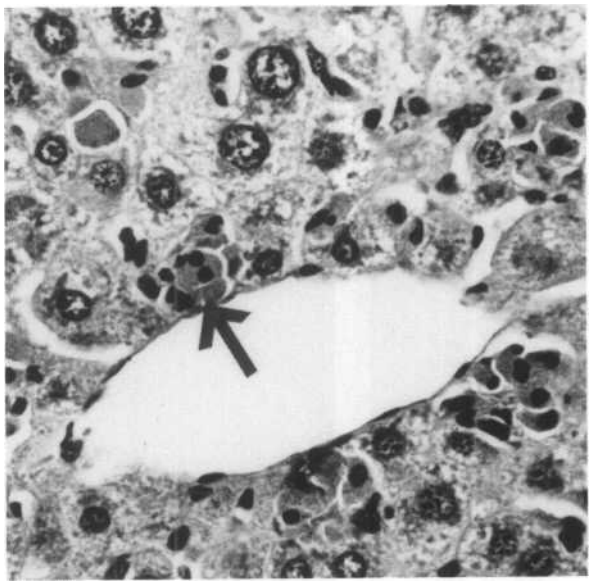


PLATE 3
Liver of a female Swiss (CD-1®) mouse given 640 mg rifabutin per kg body weight per day by gavage for 4 weeks showing mixed cellular infiltration around central vein. Phagocytic cells contain intracytoplasmic pigment (arrow). H&E; 400 x

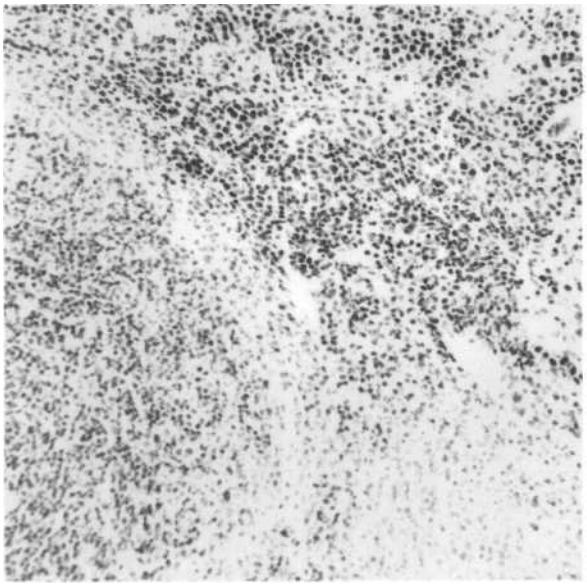


PLATE 4
Spleen of a vehicle control male Swiss (CD-1®) mouse showing no lesions. H&E; 160 X

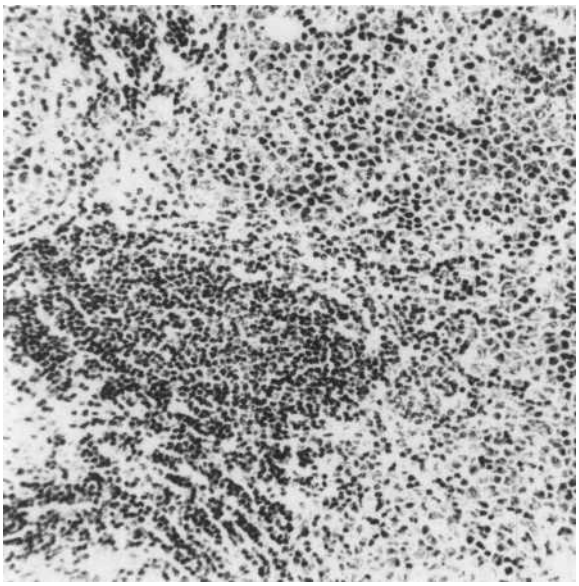


PLATE 5
Spleen of a male Swiss (CD-1®) mouse given 400 mg AZT + 640 mg rifabutin per kg body weight per day by gavage for 3 weeks showing hematopoietic cell proliferation. H&E; 160 x

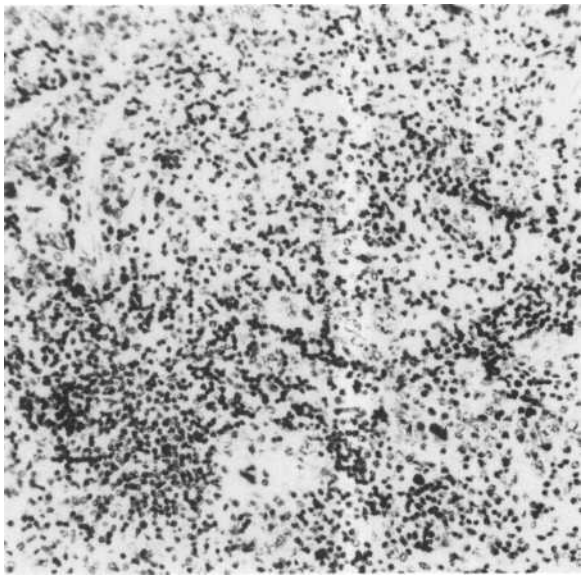


PLATE 6
Spleen of a male Swiss (CD-1®) mouse given 400 mg AZT + 640 mg rifabutin per kg body weight per day by gavage for 3 weeks showing atrophy of red pulp and lymphoid follicles. H&E; 160 x

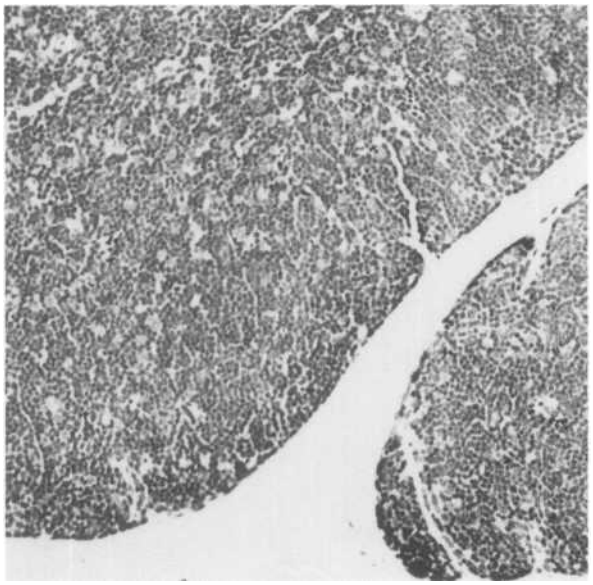


PLATE 7
Thymus of a vehicle control male Swiss (CD-I®) mouse showing no lesions. H&E; 160 x

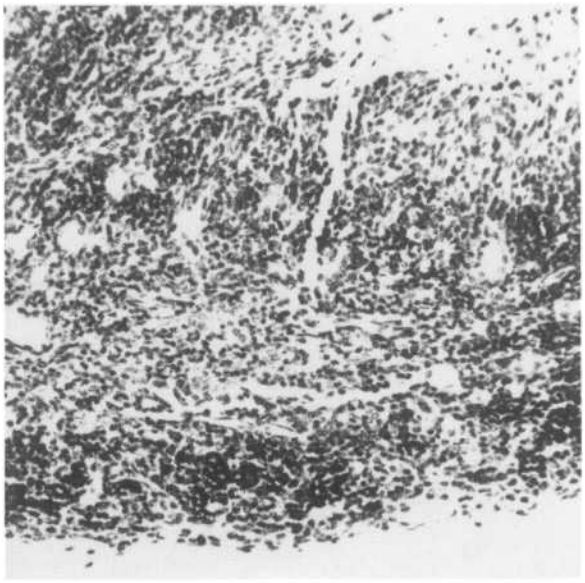


PLATE 8
Thymus of a male Swiss (CD-I®) mouse given 400 mg AZT + 640 mg rifabutin per kg body weight per day by gavage for 3 weeks showing thymic atrophy. H&E; 160 x

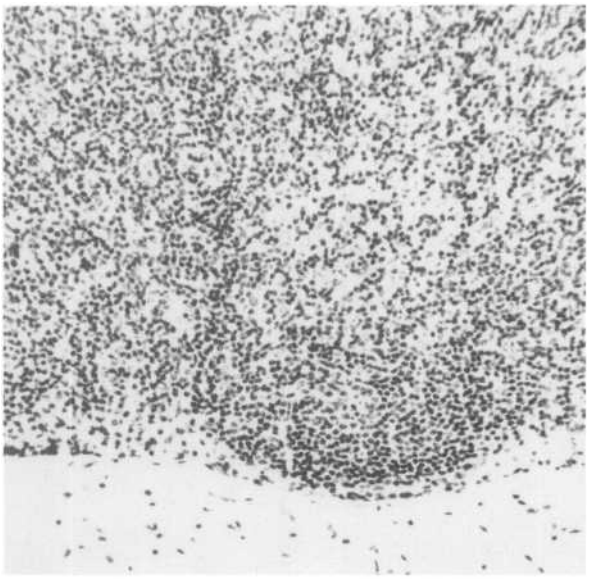


PLATE 9
Mesenteric lymph node of a vehicle control male Swiss (CD-1®) mouse showing no lesions. H&E; 160 x

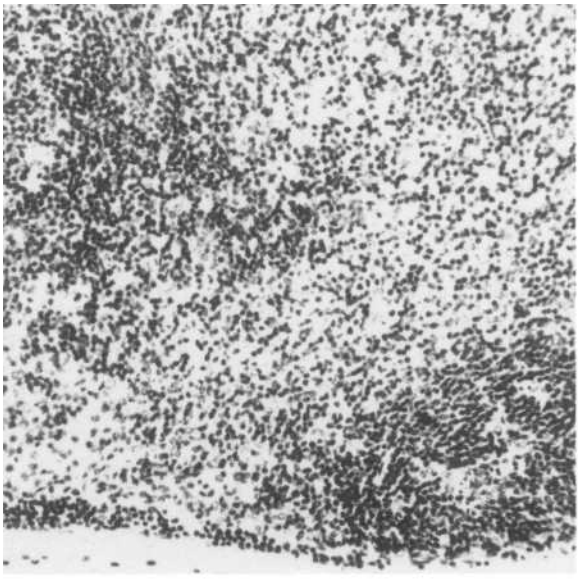


PLATE 10
Mesenteric lymph node of a male Swiss (CD-1®) mouse given 400 mg AZT + 640 mg rifabutin per kg body weight per day by gavage for 3 weeks showing atrophy of lymphoid cells. H&E; 160 x

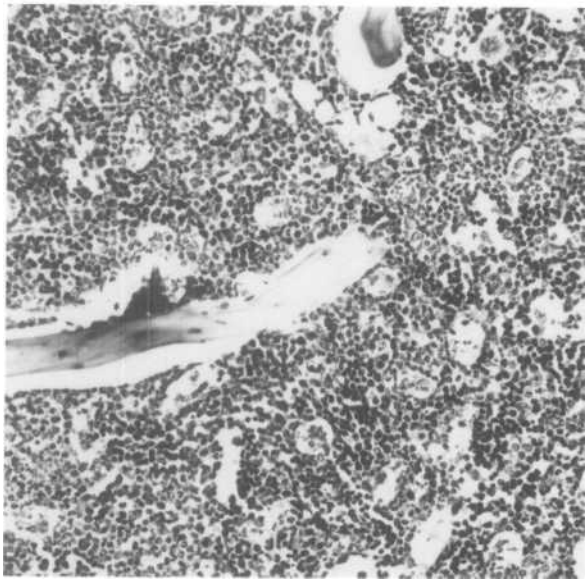


PLATE 11
Bone marrow of a vehicle control male Swiss (CD-1®) mouse showing no lesions. H&E; 160 x

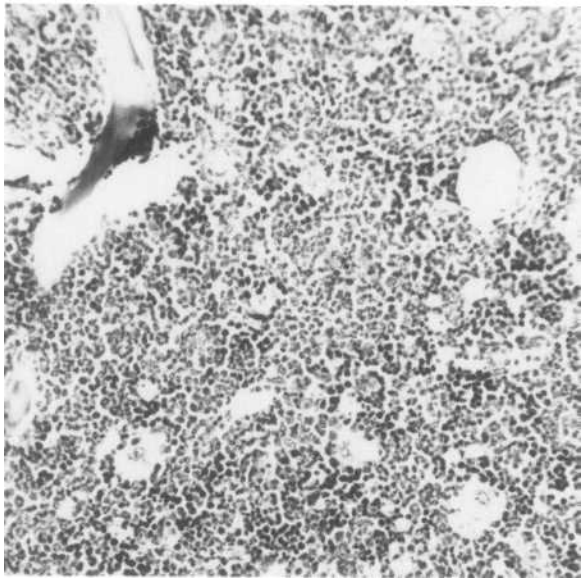


PLATE 12
Bone marrow of a male Swiss (CD-1®) mouse given 640 mg rifabutin per kg body weight per day by gavage for 3 weeks showing myeloid cell hyperplasia. H&E; 160 x

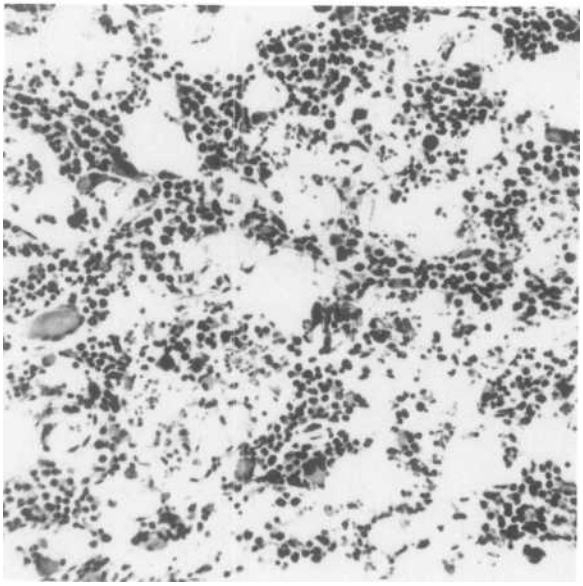


PLATE 13
Bone marrow of a male Swiss (CD-1®) mouse given 400 mg AZT + 640 mg rifabutin per kg body weight per day by gavage for 3 weeks showing depletion of erythroid cells. H&E; 160 x

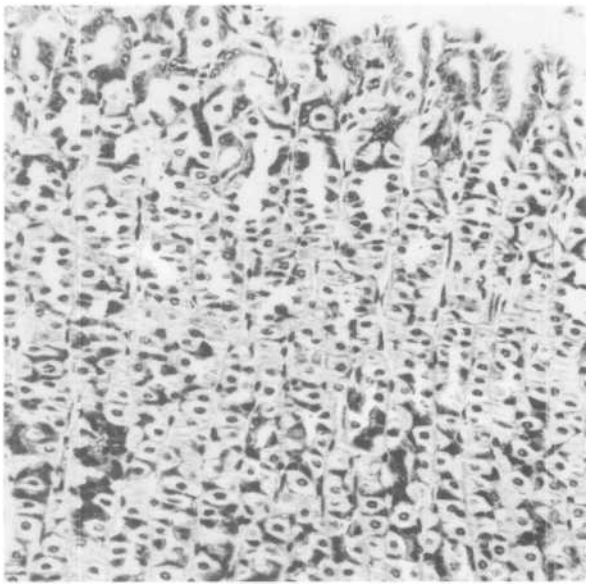


PLATE 14
Glandular stomach of a vehicle control male Swiss (CD-1®) mouse showing no lesions. **H&E;** 160 x

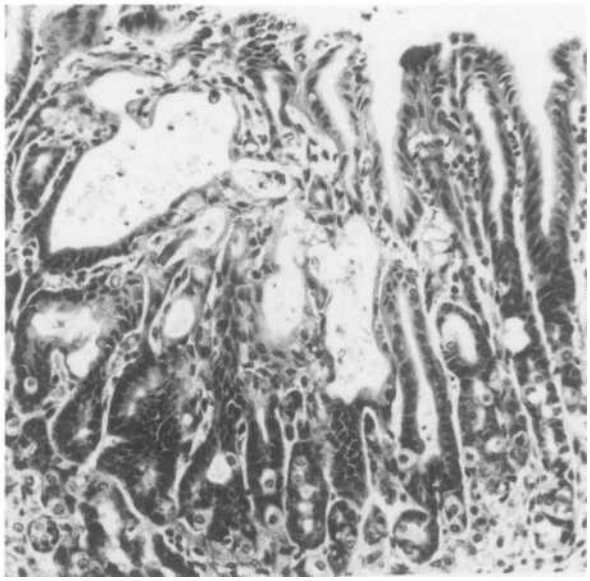


PLATE 15
Glandular stomach of a male Swiss (CD-1®) mouse given 640 mg rifabutin per kg body weight per day by gavage for 3 weeks showing cystic degeneration. H&E; 160 x

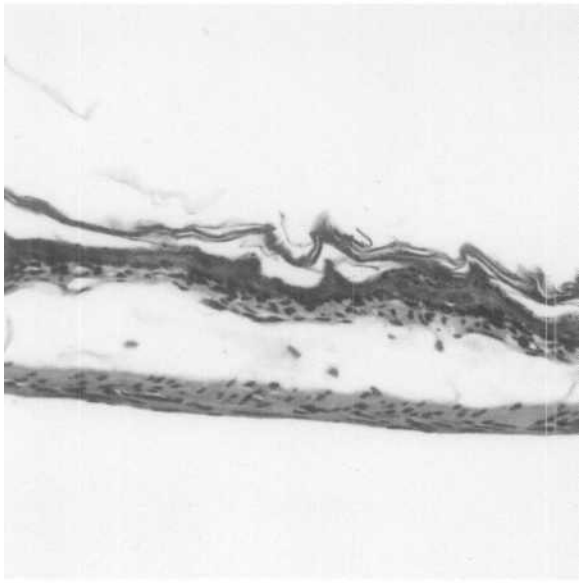


PLATE 16
Forestomach of a vehicle control male Swiss (CD-1®) mouse showing no lesions. H&E; 160 x

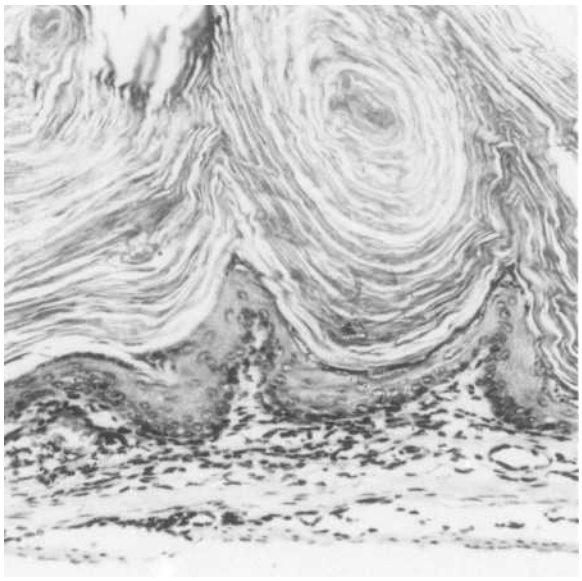


PLATE 17
Forestomach of a male Swiss (CD-1®) mouse given 400 mg AZT + 640 mg rifabutin per kg body weight per day by gavage for 3 weeks showing epithelial hyperplasia and chronic active inflammation. H&E; 160 x

Marked - approximately 91% to 100% of hepatocytes with cytoplasmic alteration

Although hepatocellular cytoplasmic alteration was seen in male and female vehicle control mice, the incidences were increased in male and female mice receiving 200 or 400 mg/kg AZT. The increased incidences of hepatocellular cytoplasmic alteration were not dose related in male or female mice.

A treatment-related increase in the incidences of splenic hematopoietic cell proliferation was seen in male mice (spleen was not examined histopathologically in the female-A mice),although the incidences were not dose related. Splenic hematopoietic cell proliferation was graded for severity based on the following criteria:

Minimal - approximately 15% or less of red pulp occupied by hematopoietic cells

Mild - approximately 16% to 50% of red pulp occupied by hematopoietic cells

Moderate - approximately 51% to 90% of red pulp occupied by hematopoietic cells

Marked - approximately 91% to 100% of red pulp occupied by hematopoietic cells

TABLE 6
Incidence and Mean Severity of Histopathologic Alterations in the Liver of Male and Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations

	Lesion[b]					
	Cytoplasmic Alteration		Mixed Cellular Infiltration		Centrilobular Atrophy	
Dose[a]	Male[c]	Female-A[d]	Male[c]	Female-A[d]	Male[c]	Female-A[d]
0 + 0	1 (2.0)	3 (2.0)	0	0	0	0
0 + 80	5 (2.6)	8 (2.3)	0	0	0	0
0 + 320	5 (2.8)	9 (2.6)	1 (2.0)	7 (1.0)	0	0
0 + 640	7 (2.4)	4 (2.5)	9 (1.2)	16 (1.5)	0	0
200 + 0	7 (2.1)	8 (2.1)	0	0	0	0
200 + 80	4 (2.3)	12 (2.3)	0	0	0	0
200 + 320	6 (2.7)	6 (3.2)	0	10 (1.0)	0	5 (1.8)
200 + 640	8 (2.3)	20 (3.0)	5 (1.2)	0	0	17 (1.5)
400 + 0	5 (2.2)	5 (2.6)	0	0	0	0
400 + 80	6 (2.3)	7 (2.9)	0	1 (1.0)	0	0
400 + 320	6 (2.3)	15 (3.0)	1 (1.0)	0	0	13 (1.8)
400 + 640	10 (3.0)	16 (3.0)	10 (1.3)	2 (1.0)	5 (1.8)	14 (1.9)

[a] Daily gavage doses of AZT + rifabutin (mg/kg per day)
[b] Incidence (number of animals with lesion) followed by the mean severity (which is based on the numeric scale of 1=minimal, 2=mild, 3=moderate, 4=marked) for the animals with the lesion
[c] n=10
[d] n=20

TABLE 7
Statistical Analysis of Mean Severity of Hepatocyte Cytoplasmic Alteration in the Liver of Male and Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations

Dose[a]	Mean Severity[b]	
	Male[c]	Female-A[d]
0 + 0	0.20 ± 0.20	0.30 ± 0.16
0 + 80	1.30 ± 0.47	0.90 ± 0.26
0 + 320	1.40 ± 0.50	1.15 ± 0.31*
0 + 640	1.70 ± 0.40*	0.50 ± 0.24
200 + 0	1.50 ± 0.34	0.85 ± 0.24
200 + 80	0.90 ± 0.38	1.35 ± 0.26
200 + 320	1.60 ± 0.48	0.95 ± 0.34
200 + 640	1.80 ± 0.33	2.95 ± 0.15**
400 + 0	1.10 ± 0.38	0.65 ± 0.26
400 + 80	1.40 ± 0.40	1.00 ± 0.32
400 + 320	1.40 ± 0.43	2.25 ± 0.33**
400 + 640	3.00 ± 0.15**	2.40 ± 0.32**

* Significantly different ($P \leq 0.05$) from the vehicle control group by Jonckheere's, Williams', or Dunnett's tests
** $P \leq 0.01$
[a] Daily gavage doses of AZT + rifabutin (mg/kg per day)
[b] Data are presented as mean ± standard error for all animals in the dose group; mean severity is based on the numeric scale of 1=minimal, 2=mild, 3=moderate, 4=marked
[c] n=10
[d] n=20

The splenic hematopoiesis involved primarily the erythropoietic cells and may have been a compensatory response to mild anemia in the AZT-treated mice. Incidences of the splenic alterations are listed in Table 8, and results of statistical analysis are presented in Table 9 and illustrated graphically in Figure 15.

Rifabutin Alone

Treatment-related lesions were seen in the liver, glandular stomach, bone marrow, spleen, and lung of male mice and in the liver, forestomach, glandular stomach, and thymus of female-A mice (bone marrow, spleen, and lung were not examined histopathologically in female mice).

Treatment-related liver alterations consisted of hepatocellular cytoplasmic alteration and mixed inflammatory cell infiltrate in male and female-A mice (Tables 6 and 7). The morphologic appearance and the criteria for severity grading of cytoplasmic alteration were similar to those described for mice treated with AZT. Although hepatocellular cytoplasmic alteration was seen in male and female vehicle control mice, the incidences of this

TABLE 8
Incidence and Mean Severity of Histopathologic Alterations in the Spleen of Male Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations

Dose[a]	Lesion[b]			
	Hematopoietic Cell Proliferation	Lymphoid Follicle Atrophy	Red Pulp Atrophy	Pigmentation
0 + 0	0	0	0	0
0 + 80	3 (1.7)	0	0	0
0 + 320	10 (2.5)	0	0	0
0 + 640	10 (2.7)	0	0	0
200 + 0	8 (2.0)	0	0	0
200 + 80	8 (1.8)	0	0	0
200 + 320	10 (2.6)	0	0	5 (2.0)
200 + 640	6 (3.2)	2 (1.5)	3 (2.0)	4 (2.3)
400 + 0	7 (1.9)	0	0	0
400 + 80	9 (2.3)	0	0	0
400 + 320	7 (3.0)	0	2 (2.0)	2 (2.5)
400 + 640	2 (4.0)	5 (2.2)	8 (2.1)	8 (2.6)

[a] Daily gavage doses of AZT + rifabutin (mg/kg per day)
[b] Incidence (number of animals with lesion; n=10) followed by the mean severity (which is based on the numeric scale of 1=minimal; 2=mild; 3=moderate; 4=marked) for the animals with the lesion

TABLE 9
Statistical Analysis of Mean Severity of Splenic Hematopoietic Cell Proliferation in Male Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations

Dose[a]	Mean Severity[b]
0 + 0	0.00
0 + 80	0.50 ± 0.27
0 + 320	2.50 ± 0.17**
0 + 640	2.70 ± 0.15**
200 + 0	1.60 ± 0.34
200 + 80	1.40 ± 0.31
200 + 320	2.60 ± 0.16
200 + 640	1.90 ± 0.59
400 + 0	1.30 ± 0.34
400 + 80	2.10 ± 0.31
400 + 320	2.10 ± 0.50
400 + 640	0.80 ± 0.53

** Significantly different from the control group ($P \leq 0.01$) by Jonckheere's and Williams' or Dunnett's tests
[a] Daily gavage doses of AZT + rifabutin (mg/kg per day)
[b] Data are presented as mean ± standard error for all animals in the dose group; mean severity is based on the numeric scale of 1=minimal, 2=mild, 3=moderate, 4=marked; n=10

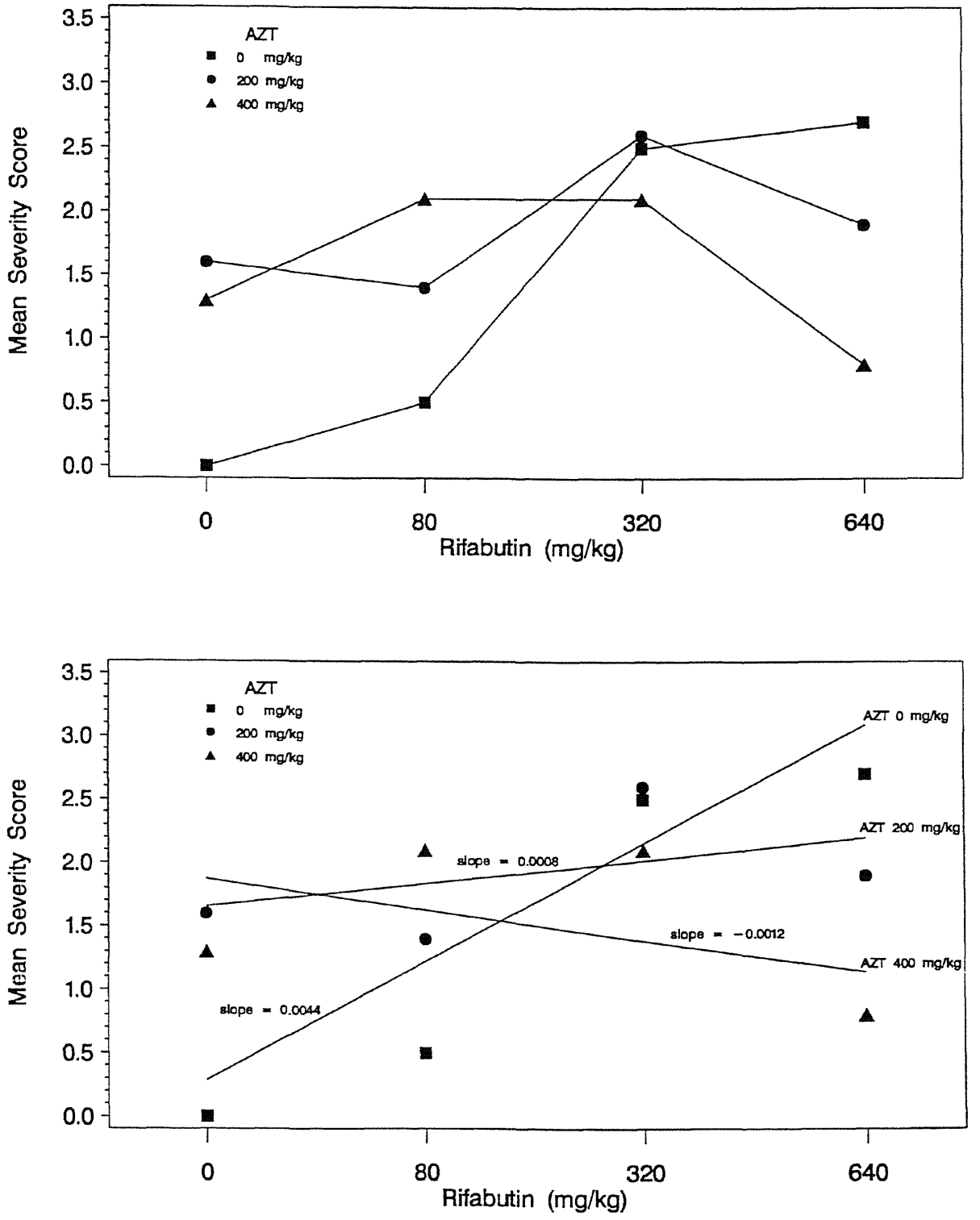


FIGURE 15
Severity of Splenic Hematopoietic Cell Proliferation Versus Dose in Male Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations

lesion were increased in all three rifabutin-treated groups compared with the vehicle control groups. The incidences of cytoplasmic alteration were dose related in male mice but not in female-A mice. As with the mice treated with AZT alone, this morphologic alteration in the cytoplasm of hepatocytes likely represents a physiologic response to decreased feed consumption and concurrent body weight loss instead of a direct toxic response to rifabutin.

Mixed cellular infiltration in the liver consisted of macrophages, small mononuclear cells, and occasional neutrophils within the liver parenchyma, with the heaviest aggregations often occurring adjacent to the interlobular and central veins. Mixed cellular infiltration was graded for severity based on the following criteria:

Minimal - up to approximately 20 mixed inflammatory cells in any 100× magnification field with inflammatory cells confined almost exclusively to perivascular areas

Mild - approximately 21 to 50 mixed inflammatory cells in any 100× magnification field with inflammatory cells primarily bordering blood vessels but occasionally occurring throughout liver parenchyma

Moderate - approximately 51 to 150 mixed inflammatory cells in any 100× magnification field with inflammatory cells occurring in significant numbers throughout the liver parenchyma as well as in perivascular areas.

The infiltrating macrophages frequently contained a reddish-brown homogeneous to finely granular intracytoplasmic pigment. The pigment was not identified, but it may have represented macrophage-sequestered rifabutin or a metabolite. The hepatic mixed cellular infiltration may have represented a host defense mechanism whereby rifabutin and/or metabolites were phagocytized and eliminated by inflammatory cells.

A compound-related glandular stomach lesion seen in male and female mice was designated "cystic degeneration" (Table 10), which consisted of varying numbers of dilated mucosal glands appearing as mucosal cysts. The mucosal cysts often contained cellular detritus and amorphous proteinaceous material, and the surrounding glandular epithelium varied in appearance from hyperplastic or hypertrophied to flattened or squamous.

TABLE 10
Incidence and Mean Severity of Histopathologic Alterations in the Glandular Stomach of Male and Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations

Dose[a]	Lesion[b]			
	Cystic Degeneration		Chronic Active Inflammation	
	Male	Female-A	Male	Female-A
0 + 0	0/10	NA	0/10	NA
0 + 640	10/10 (2.6)	16/17 (2.5)	1/10 (2.0)	11/17 (2.2)
200 + 320	1/1 (3.0)	NA	0/1	NA
200 + 640	10/10 (2.8)	20/20 (3.3)	3/10 (2.0)	4/20 (2.0)
400 + 320	1/1 (2.0)	NA	0/1	NA
400 + 640	10/10 (3.0)	20/20 (3.2)	1/10 (3.0)	7/20 (2.0)

NA=Not examined (tissues were not required to be examined unless gross lesions were present)
[a] Daily gavage doses of AZT + rifabutin (mg/kg per day); AZT alone, other doses of rifabutin alone, and AZT + 80 mg/kg rifabutin did not cause histopathologic alterations in the stomach
[b] Incidence (number of animals with lesion) followed by the mean severity (which is based on the numeric scale of 1=minimal; 2=mild; 3=moderate; 4=marked) for the animals with the lesion

Cystic degeneration of the glandular stomach was graded for severity based on the following criteria:

Minimal - a few dilated mucosal glands that do not exceed approximately 50 μm in diameter

Mild - dilatation of approximately 50 to 100 μm involving up to approximately 5% of mucosal glands

Moderate - dilatation of approximately 101 to 300 μm involving up to approximately 10% of mucosal glands

Marked - dilatation of approximately 301 to 500 μm or greater involving up to approximately 30% of mucosal glands

In the female mice, and less frequently in the males, chronic active inflammation (Table 10), characterized by infiltration of neutrophils, lymphocytes, and macrophages, was present within the mucosa and submucosa of the glandular stomach. Chronic active inflammation of the glandular stomach was graded for severity based on the following criteria:

Minimal - up to approximately 20 inflammatory cells in any 100× magnification field (considered background change and not diagnosed)

Mild - approximately 21 to 100 inflammatory cells in any 100× magnification field

Moderate - approximately 101 to 500 inflammatory cells in any 100× magnification field

Faintly staining, reddish-brown pigment similar to that seen in liver macrophages was occasionally visible within the cytoplasm of the infiltrating macrophages.

Compound-related forestomach epithelial hyperplasia (Table 11) was observed in both male and female mice but was more prominent in females. Epithelial hyperplasia of the forestomach was graded for severity based on the following criteria:

Minimal - mucosal thickness (including keratin layer) of approximately 60 to 200 μm

Mild - mucosal thickness of approximately 201 to 500 μm

Moderate - mucosal thickness of approximately 501 to 1,000 μm

Marked - mucosal thickness of approximately 1,001 μm or greater

The forestomach hyperplasia in one female mouse that received 640 mg/kg rifabutin was accompanied by chronic active inflammation (Table 11), which was also considered to be compound related. Criteria for severity grading were similar to those used for inflammation of the glandular stomach.

TABLE 11
Incidence and Mean Severity of Histopathologic Alterations in the Forestomach of Male and Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin

Dose[a]	Lesion[b]					
	Epithelial Hyperplasia		Ulcer		Chronic Active Inflammation	
	Male	Female-A	Male	Female-A	Male	Female-A
0 + 0	0/10	NA	0/10	NA	0/10	NA
0 + 640	0/10	4/17 (1.8)	0/10	0/17	0/10	1/17 (2.0)
200 + 320	NA	NA	NA	NA	NA	NA
200 + 640	2/10 (2.0)	17/20 (3.2)	0/10	12/20 (3.5)	1/10 (2.0)	6/20 (2.7)
400 + 320	1/2 (3.0)	NA	1/2 (2.0)	NA	0/2	NA
400 + 640	6/10 (2.7)	16/20 (3.1)	1/10 (4.0)	11/20 (3.5)	4/10 (2.3)	4/20 (2.3)

NA=Not examined (tissues were not required to be examined unless gross lesions were present)
[a] Daily gavage doses of AZT + rifabutin (mg/kg per day); AZT alone, other doses of rifabutin alone, and AZT + 80 mg/kg rifabutin did not cause histopathologic alterations in the stomach
[b] Incidence (number of animals with lesion) followed by the mean severity (which is based on the numeric scale of 1=minimal; 2=mild; 3=moderate; 4=marked) for the animals with the lesion

Hematopoietic cell proliferation in the spleen (Table 8), myeloid cell hyperplasia in the bone marrow (Table 12), and mixed cellular infiltration in the lung (Table 13) were seen in male mice (spleen, bone marrow, and lung were not evaluated in female mice). The criteria for severity grading of splenic hematopoietic cell proliferation (Table 9, Figure 15) were similar to that described for mice treated with AZT. The splenic hematopoietic cell proliferation may have been a compensatory response to a mild anemia. Bone marrow myeloid cell hyperplasia (Table 12, Figure 16) was graded for severity based on the following:

Minimal - up to an approximate 10% increase over normal in numbers of myeloid cells

Mild - an approximate 11% to 20% increase over normal in numbers of myeloid cells

Mixed cell infiltration of the lung was graded for severity based on the following criteria:

Minimal - up to approximately 20 inflammatory cells in any 100× magnification field

Mild - approximately 21 to 50 inflammatory cells in any 100× magnification field

Moderate - approximately 51 to 150 inflammatory cells in any 100× magnification field

TABLE 12
Incidence, Mean Severity, and Statistical Analysis of Mean Severity of Histopathologic Alterations in the Bone Marrow of Male Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations

Dose[a]	Lesion[b]			
	Myeloid Hyperplasia		Erythroid Cell Depletion	
0 + 320	1 (1.0)		0	0.00
0 + 640	6 (2.0)		0	0.00
200 + 320	1 (1.0)		3 (1.0)	0.30 ± 0.15
200 + 640	3 (2.0)		5 (2.4)	1.20 ± 0.42**
400 + 320	0		2 (2.0)	0.40 ± 0.27
400 + 640	1 (3.0)		9 (2.4)	2.20 ± 0.33**

** Significantly different ($P \leq 0.01$) from the control group by Jonckheere's and Williams' or Dunnett's tests
[a] Daily gavage doses of AZT + rifabutin (mg/kg per day)
[b] Incidence (number of animals with lesion; n=10) followed by the mean severity (which is based on the numeric scale of 1=minimal; 2=mild; 3=moderate; 4=marked) for the animals with the lesion; data in the last column are presented as mean ± standard error, with mean severity for all animals in the dose group

TABLE 13
Incidence and Mean Severity of Mixed Cellular Infiltration in the Lung of Male Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations

Dose[a]	Mixed Cellular Infiltration[b]
0 + 0	0
0 + 80	0
0 + 320	10 (1.6)
0 + 640	10 (2.1)
200 + 0	0
200 + 80	1 (1.0)
200 + 320	10 (1.8)
200 + 640	10 (2.1)
400 + 0	0
400 + 80	0
400 + 320	10 (1.7)
400 + 640	10 (1.5)

[a] Daily gavage doses of AZT + rifabutin (mg/kg per day)
[b] Incidence (number of animals with lesion; n=10) followed by the mean severity (which is based on the numeric scale of 1=minimal; 2=mild; 3=moderate; 4=marked) for the animals with the lesion

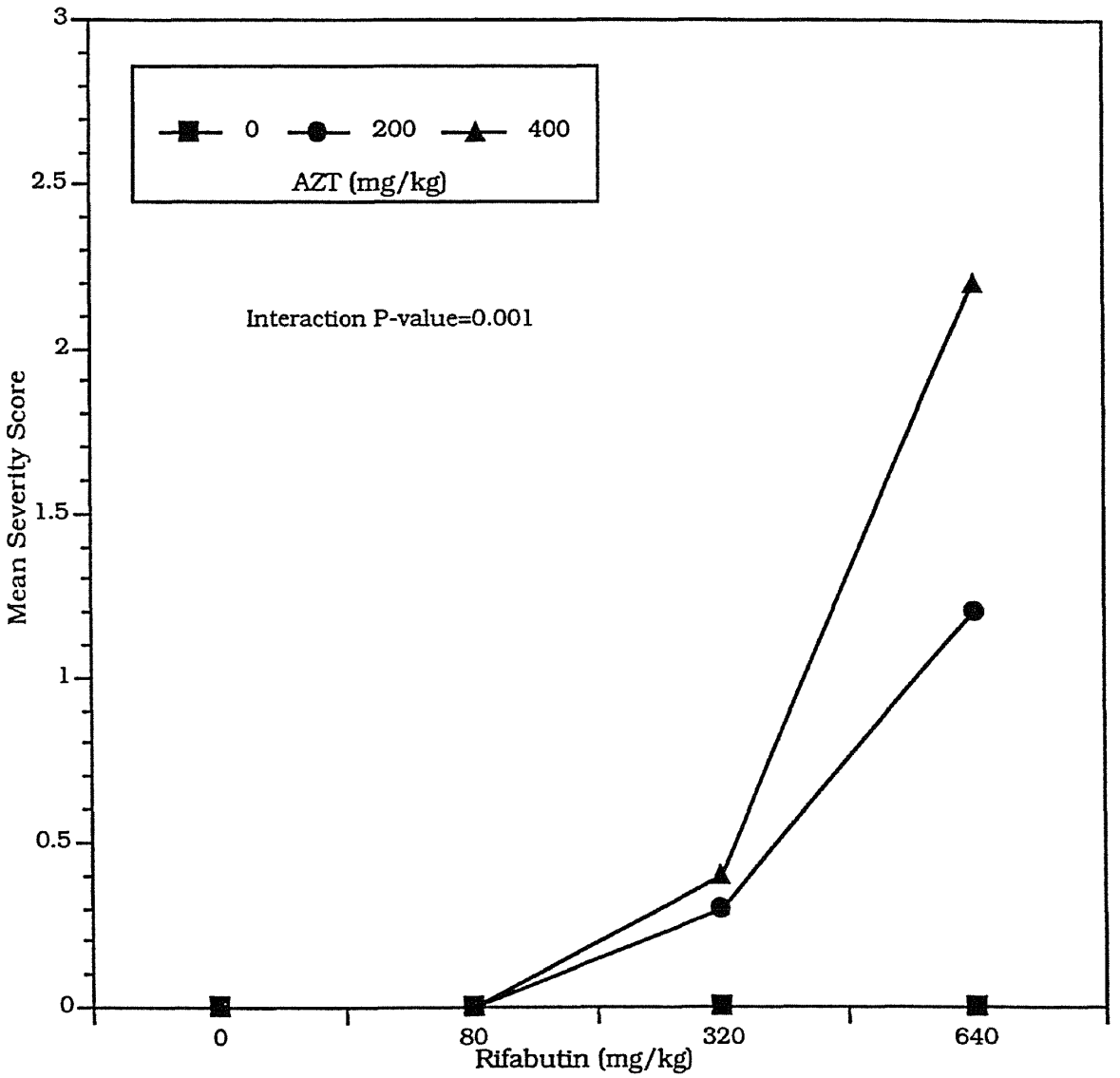


FIGURE 16
Severity of Bone Marrow Erythroid Cell Depletion Versus Dose in Male Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations

Mixed cell infiltration of the lung consisted of the presence of macrophages with fewer numbers of small mononuclear cells and neutrophils occurring singularly or in aggregates within the alveolar spaces. Most of the infiltrating macrophages contained a reddish-brown, amorphous, intracytoplasmic pigment similar in appearance to that observed within macrophages in the liver and stomach. Mixed cellular infiltration of the lung may have been a host defense mechanism against rifabutin and/or metabolites.

Thymic atrophy (Table 14) was observed consistently in female-A mice but not in males and was considered to be treatment related. The lesion was graded for severity based on the following criteria:

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

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

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

Marked - depletion of approximately 51% or greater of cortical lymphocytes

Although thymic atrophy was considered to be treatment related in female-A mice, the possibility that some of the effect may have been secondary to stress factors such as pregnancy was not discounted.

With the exception of hepatocellular cytoplasmic alteration in female mice, the incidences of all lesions attributed to rifabutin administration were dose related.

AZT and Rifabutin

Treatment-related lesions were seen in the liver (Tables 6, 7, and 14), spleen (Tables 8 and 9), glandular stomach (Table 10), forestomach (Table 11), bone marrow (Figure 16 and Table 12), lung (Table 13), thymus (Table 15), and mesenteric lymph node of male mice. Treatment-related lesions in female mice were seen in the liver (Tables 6, 7, and 14), glandular stomach (Table 10), forestomach (Table 11), and thymus (Table 15). Spleen, bone marrow, lung, and lymph nodes were not evaluated in female mice. In male mice, coadministration of 400 mg of AZT with rifabutin at 640 mg and not at 320 mg appeared to have markedly increased the bone marrow erythroid cell depletion (Figure 16). Since rifabutin alone had no effect on bone marrow erythroid cell depletion, there appears to be an interaction to enhance the bone marrow toxicity of AZT by rifabutin at higher doses. In general, the lesions caused by combination treatment with AZT and rifabutin in various tissues were similar to but more pronounced than those caused by treatment with AZT or rifabutin alone.

TABLE 14
Statistical Analysis of Mean Severity of Hepatocellular Centrilobular Atrophy in Male and Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin in Combinations

Dose[a]	Mean Severity[b]	
	Male[c]	Female-A[d]
0 + 0	0 00	0.00
200 + 80	0 00	0.00
200 + 320	0 00	0.45 ± 0 19*
200 + 640	0.00	1.30 ± 0 16**
400 + 80	0 00	0.00
400 + 320	0.00	1.20 ± 0 23**
400 + 640	0.90 ± 0.31**	1.30 ± 0 23**

* Significantly different ($P \leq 0.05$) from the vehicle control group by Jonckheere's and Williams' or Dunnett's tests
** $P \leq 0$ 01
[a] Daily gavage doses of AZT + rifabutin (mg/kg per day), AZT alone or rifabutin alone did not cause centrilobular atrophy
[b] Data are presented as mean ± standard error for all animals in the dose group; mean severity is based on the numeric scale of 1=minimal, 2=mild, 3=moderate, 4=marked
[c] n=10
[d] n=20

TABLE 15
Incidence, Mean Severity, and Statistical Analysis of Mean Severity of Thymic Atrophy in Male and Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations

Dose[a]	Atrophy[b]					
	Male			Female-A		
0 + 320	1/10 (1.0)	0.10 ± 0.10	10	N/A	0 00	20
0 + 640	0/10	0 00	9	10/16 (2.5)	1.25 ± 0.31**	20
200 + 320	4/10 (1.5)	0.60 ± 0.31	10	N/A	0.00	20
200 + 640	6/9 (1.5)	0.90 ± 0.31**	10	20/20 (3.0)	3.00 ± 0.07**	20
400 + 320	6/9 (1.0)	0.60 ± 0.16	10	N/A	0.00	20
400 + 640	6/6 (2.5)	1.50 ± 0.43**	10	20/20 (3.2)	3.20 ± 0.12**	20

NA=Not examined (tissue was not saved unless a gross lesion was present)
** Significantly different ($P \leq 0.01$) from the control group by Jonckheere's and Williams' or Dunnett's tests
[a] Daily gavage doses of AZT + rifabutin (mg/kg per day); AZT alone and rifabutin at 80 mg/kg alone or in combination with AZT did not cause thymic atrophy
[b] Incidence (number of animals with lesion/number of animals examined) followed by the mean severity (which is based on the numeric scale of 1=minimal; 2=mild; 3=moderate; 4=marked) for the animals with the lesion (second and fifth columns); data in the third and sixth columns are presented as mean ± standard error, with mean severity for all animals in the dose group; data in the fourth and seventh columns are the number of animals examined

Compound-related liver lesions seen in male and female-A mice included hepatocellular cytoplasmic alteration, mixed cellular infiltration, and centrilobular atrophy. Compound-related liver lesions seen in female mice only consisted of centrilobular necrosis. Cytoplasmic alteration and mixed cellular infiltration were graded for severity using the same criteria as that used in evaluating tissues from mice treated with AZT or rifabutin alone. Centrilobular atrophy was graded for severity based on the following criteria:

Minimal - atrophy or necrosis of approximately 5% or less of centrilobular hepatocytes

Mild - atrophy or necrosis of approximately 6% to 15% of centrilobular hepatocytes

Moderate - atrophy or necrosis of approximately 16% to 50% of centrilobular hepatocytes

Although hepatocellular cytoplasmic alteration was seen in male and female vehicle control mice, incidences were increased in the AZT + rifabutin-treated groups (Tables 6 and 7). Incidences of cytoplasmic alteration in hepatocytes were not statistically significant in male or female mice treated with AZT alone. Rifabutin alone at 640 mg/kg in male mice resulted in a significant increase ($P \leq 0.05$) in the mean severity of cytoplasmic alteration. Female mice administered 320 mg/kg rifabutin alone had a significant increase in the incidence ($P \leq 0.05$) of hepatocyte cytoplasmic alteration, but this effect was not evident in the females treated with 640 mg/kg rifabutin alone. For male mice that received 400 + 640 mg/kg, severity of cytoplasmic alteration was enhanced ($P \leq 0.01$). Significant elevations ($P \leq 0.01$) in the severity grades of cytoplasmic alteration also occurred in female groups treated with 200 + 320 mg/kg or 400 + 640 mg/kg. The degree of severity of cytoplasmic alteration in the high dose combination female group may have been low because of early deaths occurring prior to the development of the lesion. As previously discussed in groups receiving AZT and rifabutin alone, hepatocyte cytoplasmic alteration likely represents a physiologic response to decreased food intake with concurrent loss of body weight instead of a direct cellular response to the test compounds.

Liver mixed cellular infiltration, which consisted of infiltration of small mononuclear cells, pigmented macrophages, and occasional neutrophils, was dose related in male but not in female mice. The absence of a dose-related pattern for liver mixed cell infiltration in female mice resulted from the fact that the lesion was infrequently seen in the female mice from the higher dose combination treatment groups that died early or were euthanatized moribund; it occurred primarily in mice that survived until terminal sacrifice.

Liver centrilobular atrophy, which consisted of a paucity of hepatocytes in the centrilobular areas and which was probably a sequential change that followed centrilobular necrosis, was dose related in male and female mice; the incidences in females were higher than in males, and in females, it occurred primarily in those that died or were sacrificed moribund (Table 14). Centrilobular atrophy of hepatocytes may have been a secondary response to severe anemia instead of a direct effect of the compounds on the liver parenchyma. A significant

elevation ($P \leq 0.01$) occurred in the mean severity grade of centrilobular atrophy in male mice treated with 400 + 640 mg/kg (Table 14). This morphologic alteration did not occur in any of the other male groups that received AZT or rifabutin alone or in any combination. Female mice were affected to a greater degree than males as increased incidences of centrilobular atrophy were evident in the group administered 200 + 320 mg/kg ($P \leq 0.05$) as well as the group administered 200 + 640 mg/kg ($P \leq 0.01$). Increased incidences of centrilobular atrophy also occurred in both female groups receiving 400 mg/kg AZT + 320 or 640 mg/kg rifabutin ($P \leq 0.01$). The degree of severity appeared to reach a plateau around 1.3 in the high dose combination group because of the early deaths in this group. Centrilobular atrophy did not occur in female groups treated with AZT or rifabutin alone. Treatment-related centrilobular necrosis was seen in a few female mice, although the incidence was not dose related.

Treatment-related forestomach lesions seen in male and female-A mice consisted of epithelial hyperplasia, ulcer, and chronic active inflammation (Table 11). Female mice treated with 200 or 400 mg/kg AZT + 640 mg/kg rifabutin had significant elevations ($P \leq 0.01$) in severities of epithelial hyperplasia of the forestomach. The incidence of epithelial hyperplasia was also significant ($P \leq 0.01$) in the male group administered 400 + 640 mg/kg. Compound-related glandular stomach lesions seen in male and female mice consisted of cystic degeneration and chronic active inflammation (Table 10). The morphologic appearance and criteria for severity grading of the forestomach and glandular stomach lesions seen in AZT + rifabutin-treated mice were similar to those described for mice treated with rifabutin alone. Forestomach ulcer was graded for severity based on the following criteria:

Minimal - area of mucosal ulceration up to approximately 300 μm in diameter

Mild - area of mucosal ulceration approximately 301 to 600 μm in diameter

Moderate - area of mucosal ulceration approximately 601 to 1,200 μm in diameter

Marked - area of mucosal ulceration approximately 1,201 μm or greater in diameter

Treatment-related thymic atrophy occurred in male and female-A mice (Table 15), whereas the following lesions were seen in male mice only (the tissues involved were not evaluated in female mice): bone marrow erythroid cell depletion, bone marrow myeloid cell hyperplasia, mesenteric lymph node atrophy, splenic lymphoid follicle atrophy, splenic red pulp atrophy, splenic hematopoietic cell proliferation, splenic pigmentation, and lung mixed cell infiltration. The morphologic appearance and criteria for severity grading of the thymic atrophy, bone marrow myeloid hyperplasia, splenic hematopoietic cell proliferation, and lung mixed cellular infiltration seen in AZT + rifabutin-treated mice were similar to those described for mice treated with rifabutin alone.

Erythroid cell depletion in the bone marrow corresponding with severe anemia was evident in male mice receiving 200 or 400 mg/kg AZT + 640 mg/kg rifabutin (Table 12, Figure 16). Bone marrow erythroid cell depletion was graded for severity based on the following criteria:

Minimal - depletion of approximately 5% or less of erythroid cells

Mild - depletion of approximately 6% to 20% of erythroid cells

Moderate - depletion of approximately 21% to 50% of erythroid cells

Atrophy of the mesenteric lymph node was evident in 40% of male mice treated with 200 or 400 mg/kg AZT + 640 mg/kg rifabutin.

Splenic hematopoiesis, which may have been a compensatory response to anemia, was apparently negated in the higher dose combination groups by the atrophic effect of treatment on the splenic red pulp. The splenic pigmentation consisted of coarse, golden-brown granules that resembled hemosiderin within macrophages in the red pulp. Most of the macrophages that contributed to the mixed cell infiltration in the lung contained a reddish-brown, amorphous pigment, which may have represented rifabutin or a metabolite of rifabutin.

Other Lesions

Lesions not attributed to administration of either compound were seen in the liver of male and female mice and in the bone, kidney, mandibular lymph node, heart, lung, glandular stomach, urethra, and skin of male mice. Liver focal necrosis was seen in male and female-A mice from the vehicle control group and various treatment groups. Although the incidences were increased in some of the treatment groups over the vehicle control, a dose-related pattern was not evident, and the increase was considered to be incidental. Liver diffuse necrosis of marked severity was seen in one female-A mouse that received 400 mg/kg AZT alone; this probably occurred as a result of an incidental torsion of the affected liver lobe. Cartilaginous metaplasia, fibrosis, and/or necrosis involving bone were seen in several male mice. These lesions, which occurred sporadically in various groups including the control group, involved the thoracic vertebrae and ribs near their junction with the vertebrae. The bone sections were examined microscopically as a result of nodules or other lesions observed on the vertebrae or ribs at necropsy, and the lesions were attributed to mechanical irritation caused by the gavage needle during dosing. Kidney lesions seen in male mice that were considered incidental included protein casts, cyst, hydronephrosis, chronic inflammation, and renal tubular regeneration. Other lesions in male mice considered to be incidental included liver hemorrhage, mandibular lymph node hemorrhage, heart mineralization, lung hemorrhage, glandular stomach hemorrhage, glandular stomach edema, urethral inflammation, urethral cyst, and skin hemorrhage.

SPERM ENDPOINTS

Epididymal sperm motility was affected by the interaction of AZT and rifabutin. There was a statistically significant interaction between both compounds, indicating that the dose-response relationship of AZT differs across doses of rifabutin ($P \leq 0.01$; Table 16). Left caudal weights were significantly decreased ($P \leq 0.01$) at 640 mg/kg rifabutin (Table 17).

TABLE 16
Effect of Oral Administration of AZT and Rifabutin on Epididymal Sperm Motility and Spermatid Heads per Testis in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations

	Epididymal Sperm Motility (%)		Spermatid Heads/Testis × 10^7	
Dose[a]	Number	Mean ± Standard Error	Number	Mean ± Standard Error
0 + 0	10	75.13 ± 5.12	10	1.82 ± 0.07
0 + 80	10	69.79 ± 4.69	10	1.86 ± 0.07
0 + 320	10	62.08 ± 7.63	10	1.86 ± 0.06
0 + 640	10	74.31 ± 2.69	10	1.78 ± 0.06
200 + 0	9	49.98 ± 7.35*	10	1.98 ± 0.05
200 + 80	9	70.36 ± 4.54	10	2.04 ± 0.08
200 + 320	9	57.57 ± 8.06	10	1.81 ± 0.10
200 + 640	9	20.71 ± 9.51**	10	1.96 ± 0.08
400 + 0	9	50.71 ± 8.05	10	1.87 ± 0.09
400 + 80	10	55.70 ± 7.72	10	1.83 ± 0.10
400 + 320	8	44.74 ± 5.58*	10	1.96 ± 0.05
400 + 640	9	3.87 ± 1.14**	10	1.93 ± 0.06

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Wilcoxon rank sum test
** $P \leq 0.01$
[a] Daily gavage doses of AZT + rifabutin (mg/kg per day)

TABLE 17
Effect of Oral Administration of AZT and Rifabutin on Left Caudal Epididymal and Left Testicular Weights in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations

Dose[a]	Left Caudal Weight (g) (Mean ± Standard Error)	Left Testicular Weight (g) (Mean ± Standard Error)
0 + 0	0.0197 ± 0.0010	0.1217 ± 0.0054
0 + 80	0.0183 ± 0.0006	0.1196 ± 0.0044
0 + 320	0.0194 ± 0.0009	0.1193 ± 0.0038
0 + 640	0.0184 ± 0.0010**	0.1203 ± 0.0046
200 + 0	0.0214 ± 0.0005	0.1174 ± 0.0042
200 + 80	0.0202 ± 0.0010	0.1278 ± 0.0065
200 + 320	0.0179 ± 0.0010	0.1189 ± 0.0042
200 + 640	0.0177 ± 0.0011**	0.1233 ± 0.0048
400 + 0	0.0199 ± 0.0006	0.1213 ± 0.0029
400 + 80	0.0209 ± 0.0009	0.1202 ± 0.0037
400 + 320	0.0195 ± 0.0008	0.1156 ± 0.0040
400 + 640	0.0171 ± 0.0011**	0.1241 ± 0.0041

** Significantly different ($P \leq 0.01$) from the vehicle control group by Jonckheere's and Williams' or Dunnett's tests
[a] Daily gavage doses of AZT + rifabutin (mg/kg per day)

NATURAL DELIVERY DATA

The administration of AZT or rifabutin alone or in combination did not affect the incidence of pregnancy in the female-B mice (Table 18). Administration of 200 + 640, 400 + 320, or 400 + 640 mg/kg AZT and rifabutin significantly ($P \leq 0.05$ or $P \leq 0.01$) decreased the number of delivered litters (Table 18). The 200 + 640 and 400 + 320 mg/kg doses significantly ($P \leq 0.05$) increased the duration of gestation.

The number of implantation sites was not affected by administration of any test article alone or in combination. The number of dams with stillborn pups, the number of dams with no liveborn pups, and the number of dams with all pups dying were increased or significantly ($P \leq 0.05$) increased in the 200 + 640 and 400 + 320 mg/kg groups.

TABLE 18
Occurrence of Pregnancy in Female-B Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations

Dose[a]	Number	Number Pregnant[b] (% of total)	Number Delivered (% of number pregnant)
0 + 0	15	15 (100)	15 (100)
0 + 80	15	11 (73.3)	11 (100)
0 + 320	17	15 (88.2)	12 (80.0)
0 + 640	16	16 (100)	16 (100)
200 + 0	15	14 (93.3)	14 (100)
200 + 80	15	14 (93.3)	14 (100)
200 + 320	16	16 (100)	14 (87.5)
200 + 640	16	14 (87.5)	4 (28.6)**
400 + 0	16	13 (81.2)	13 (100)
400 + 80	16	12 (75.0)	12 (100)
400 + 320	16	12 (75.0)	6 (50.0)**
400 + 640	16	13 (81.2)	0**

** Significantly different ($P \leq 0.05$ or $P \leq 0.01$) from the vehicle control group value by the Kruskal-Wallis test
a Daily gavage doses of AZT + rifabutin (mg/kg per day)
b Number of animals presumed pregnant by presence of a vaginal plug

Delivered litter size was decreased in the 200 + 320, 200 + 640, and 400 + 320 mg/kg groups (Table 19). The number of stillborn pups was significantly ($P \leq 0.01$) increased in the 200 + 640 mg/kg group. The number of pups found dead or missing and presumed cannibalized was significantly ($P \leq 0.01$) increased in the 200 + 640 and 400 + 320 mg/kg groups.

Cumulative survival per litter and live litter size at weighing was decreased in the 200 + 320, 200 + 640, and 400 + 320 mg/kg groups. Pup mean body weight per litter (Figure 17) was significantly ($P \leq 0.05$) decreased or decreased in the 400 mg/kg AZT + 80 or 320 mg/kg rifabutin groups.

TABLE 19
Summary of Natural Delivery Litter Data for Female-B Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations

Dose[a]	Dams with Stillborn Pups (% of Dams that Delivered)	Dams with All Pups Dying on Days 0-4 (% of Dams with Liveborn Pups)	Mean Live Litter Size	Pups Dying on Days 1-4/Total Alive on Day 1 (%)	Survival/Live Litter Size[b] on Day 4 Postpartum
0 + 0	1 (6.7)	0	11.2	3/157 (1.9)	11.0/11.2
0 + 80	0	0	11.1	7/122 (5.7)	10.4/11.1
0 + 320	0	0	10.7	2/125 (1.6)	10.2/10.7
0 + 640	0	1 (6.2)	11.9	15/190 (7.9)*	10.9/11.9
200 + 0	0	0	12.0	0/163	11.6/12.0
200 + 80	1 (7.1)	0	10.1	5/130 (3.8)	9.6/10.1
200 + 320	1 (7.1)	2 (15.4)	8.0	7/102 (6.9)	7.3/8.0
200 + 640	2 (50.0)	2 (66.7)	4.0	2/4 (50.0)**	0.7/4.0
400 + 0	1 (7.7)	0	10.7	2/137 (1.4)	10.4/10.7
400 + 80	1 (8.3)	0	11.8	6/140 (4.3)	11.2/11.8
400 + 320	3 (50.0)	2 (50.0)	6.0	2/18 (11.1)	4.0/6.0
400 + 640	—[c]	—	—	—	—

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Cochran-Armitage and Fisher exact tests
** $P \leq 0.01$
[a] Daily gavage doses of AZT + rifabutin (mg/kg per day)
[b] Excludes values for litters that had no surviving pups
[c] No litters were delivered

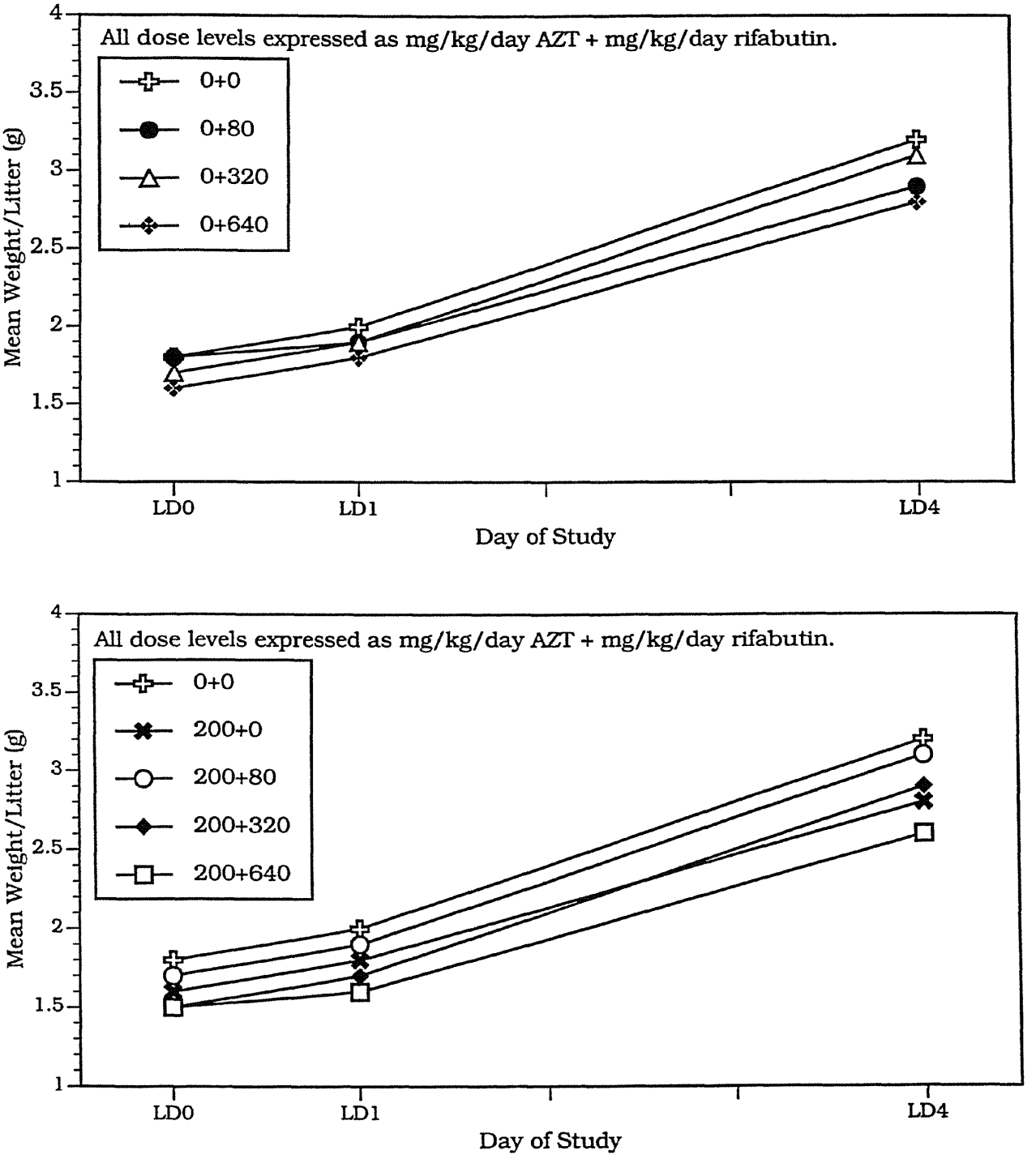


FIGURE 17
Mean Body Weights/Litter of Pups of Female-B Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations (LD=lactation day; mean pup weight/litter includes only values for litters in which pups survived to LD 4)

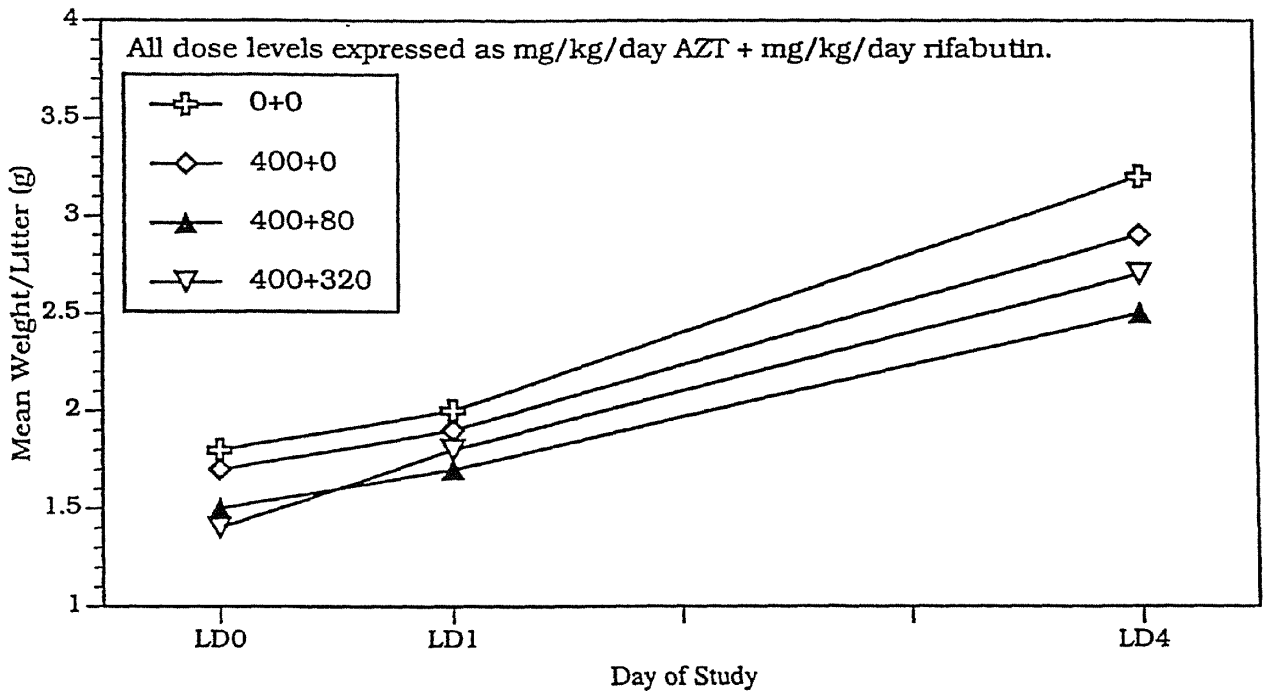


FIGURE 17
Mean Body Weights/Litter of Pups of Female-B Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations (LD=lactation day; mean pup weight/litter includes only values for litters in which pups survived to LD 4)

CAESAREAN-SECTION DATA

Pregnancy rates were significantly ($P \leq 0.05$ or $P \leq 0.01$) reduced in the following groups (Table 20): 200 mg/kg AZT alone, 640 mg/kg rifabutin alone, 200 mg/kg AZT+ 320 or 640 mg/kg rifabutin, and 400 mg/kg AZT + 80, 320, or 640 mg/kg rifabutin. For pregnant dams, the average number of corpora lutea and implantations per litter were not affected by treatment with AZT or rifabutin alone or in combination.

Live litter size was significantly ($P \leq 0.01$) reduced, and the average number of resorptions and percentage of dead or resorbed conceptuses per litter were significantly ($P \leq 0.05$ or $P \leq 0.01$) increased for the AZT alone groups and most dose groups treated with combinations (Table 21). For the average number of resorption data, only early resorptions were increased; late resorptions were not affected.

The mean fetal body weight per litter was decreased or significantly ($P \leq 0.05$ or $P \leq 0.01$) decreased in the 640 mg/kg rifabutin alone group, the AZT alone groups, and all groups treated with the combinations (Table 22).

TABLE 20
Occurrence of Pregnancy in Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations

Dose[a]	Number Pregnant (%)
0 + 0	20 (100)
0 + 80	20 (100)
0 + 320	19 (95)
0 + 640	13 (65)**
200 + 0	15 (75)*
200 + 80	16 (80)
200 + 320	10 (50)**
200 + 640	0**
400 + 0	17 (85)
400 + 80	10 (50)**
400 + 320	4 (20)**
400 + 640	0**

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Cochran-Armitage and Fisher exact tests
** $P \leq 0.01$
[a] Daily gavage doses of AZT + rifabutin (mg/kg per day)

TABLE 21
Summary of Caesarean-Section Litter Data for Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations

Dose[a]	Litter Sizes	Live Fetuses		Dead Fetuses		Resorptions	
	Mean ± SD[b]	No.	Mean ± SD	No.	Mean ± SD	No.	Mean ± SD
0 + 0[c]	11.3 ± 2.0	200	11.1 ± 2.0	3	0.2 ± 0.4	17	0.9 ± 1.2
0 + 80[d]	12.2 ± 1.9	217	12.0 ± 2.1	2	0.1 ± 0.3	9	0.5 ± 0.9
0 + 320	8.6 ± 3.6	154	8.1 ± 4.0	10	0.5 ± 2.3	27	1.4 ± 1.7
0 + 640[d]	12.2 ± 2.2	131	11.9 ± 2.2	3	0.3 ± 0.6	4	0.4 ± 0.5
200 + 0[d]	6.2 ± 3.7**	87	6.2 ± 3.7**	0	0.0	59	4.2 ± 2.6**
200 + 80[d]	6.9 ± 3.6**	103	6.9 ± 3.8**	1	0.1 ± 0.2	44	2.9 ± 2.1*
200 + 320[e]	3.4 ± 4.4**	31	3.4 ± 4.4**	0	0.0	33	3.7 ± 1.7**
200 + 640[f]	—	—	—	—	—	—	—
400 + 0	4.6 ± 4.2**	78	4.6 ± 4.1**	1	0.0 ± 0.2	82	4.8 ± 3.3**
400 + 80	4.0 ± 3.7**	38	3.8 ± 3.4**	2	0.2 ± 0.4	54	5.4 ± 3.7**
400 + 320[e]	3.3 ± 5.8	10	3.3 ± 5.8	0	0.0	14	4.7 ± 2.1
400 + 640[f]	—	—	—	—	—	—	—

* Significantly different ($P \leq 0.05$) from the vehicle control group value by the Cochran-Armitage and Fisher exact tests
** $P \leq 0.01$
a Daily gavage doses of AZT + rifabutin (mg/kg per day)
b SD=standard deviation
c Excludes dams that delivered litters before scheduled sacrifice
d Excludes dams that were sacrificed on an estimated day 13 or 14 of gestation and, due to gestational age of fetuses, genders and viability could not be determined
e Excludes dams that were moribund sacrificed on day 26 or 27 of the study
f All animals in the dose group were dead prior to scheduled caesarean-section. No pregnancies were detected in this group.

TABLE 22
Mean Body Weights of Fetuses from Female-A Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations

Dose[a]	Mean Fetal Weight (grams)
0 + 0	1.38
0 + 80	1.42
0 + 320	1.46
0 + 640	1.12
200 + 0	1.10**
200 + 80	1.12*
200 + 320	1.13
200 + 640	—[b]
400 + 0	1.06
400 + 80	1.12
400 + 320	—
400 + 640	—

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's or Dunn's test
** $P \leq 0.01$
[a] Daily gavage doses of AZT + rifabutin (mg/kg per day)
[b] No fetuses were present.

GROSS EXTERNAL ALTERATIONS

With the exception of red-colored fetuses in the groups treated with 320 or 640 mg/kg rifabutin, administration of AZT alone, rifabutin alone, or AZT in combination with rifabutin did not increase the percent of fetuses with any alteration per litter, the percent of litters with any alteration, or the percent of fetuses with any alteration.

Reproductive toxicity was observed in the mice that received the highest dose of rifabutin (640 mg/kg) alone or combinations of AZT and 320 or 640 mg/kg rifabutin. The highest dose of rifabutin alone, both doses of AZT alone, and all combination doses tested produced developmental toxicity (embryo lethality and reduced fetal weights but no external malformations). The combination of drugs markedly increased the reproductive and developmental toxicity caused by either drug alone.

Severity of hematopoietic toxicity due to AZT and rifabutin combinations was dose- and duration-of-treatment dependent. Higher dose combinations (200 or 400 mg/kg AZT + 640 mg/kg rifabutin) caused mild anemia in female-B mice treated for 10 days, severe anemia in male mice treated for 20 days, and the death of all female-A mice by day 22. These results indicate that the hematopoietic toxicity in pregnant female mice was more severe than in males and pregnancy may have contributed to the increased hematopoietic toxicity of AZT + rifabutin combinations. Cytoplasmic alterations in hepatocytes and centrilobular atrophy of the liver appear to be dose- and duration-of-treatment dependent and more severe in pregnant females than in males.

These effects may be secondary to anemia rather than a direct hepatotoxicity of the therapeutic combinations. Plasma concentrations of AZT as well as rifabutin appeared to have increased at the 400 mg/kg AZT + 600 mg/kg rifabutin combination dose when compared to the plasma concentrations of the same doses administered alone. These results indicate that there may be an interaction in absorption, metabolism, or elimination of these compounds when given in combination, especially at high doses. Increased hematopoietic toxicity of the therapeutic combinations may be due to increased plasma levels of these compounds.

Combinations of AZT at 200 and 400 mg/kg with rifabutin at 320 and 640 mg/kg caused mortality after 4 to 19 days of treatment, as well as reproductive and developmental toxicity in female-A and female-B mice. Because most reproductive and developmental toxic effects in female-B mice were observed at the doses that caused maternal toxicity, increases in reproductive and developmental toxicity at these doses may be secondary to increased maternal toxicity. Increased maternal toxicity, as measured by body weights and hematological parameters, was not observed in female-A mice treated with 400 mg/kg AZT + 80 mg/kg rifabutin as compared to the same doses of AZT or rifabutin alone. However, the above dose combination increased the reproductive and developmental toxicity. Therefore, rifabutin at 80 mg/kg, which had no reproductive or developmental effects when administered alone, appears to have increased the reproductive and developmental toxicity of AZT at 400 mg/kg.

CONCLUSIONS

AZT alone caused mild hematopoietic toxicity as indicated by anemia. Rifabutin alone caused mild hematopoietic toxicity and mild hepatic toxicity with increases in liver weights and serum enzymes indicative of liver toxicity. Rifabutin also caused mild degenerative and inflammatory lesions in the stomach. Combinations of AZT and rifabutin caused hematopoietic toxicity accompanied by severe anemia and decreased hematopoietic cells in the bone marrow and spleen. Severe anemia appeared to have contributed to high mortality of female-A mice treated with combinations of 200 or 400 mg/kg AZT and 320 or 640 mg/kg rifabutin. Combinations of the two compounds appeared to have synergized the hematopoietic toxicity leading to mortality. AZT increased the hepatic toxicity but not the gastric lesions caused by rifabutin. AZT and rifabutin at the highest dose combination evaluated in this study appear to have increased the plasma levels of both compounds as compared to administration of either compound alone, and increased plasma levels may have contributed to the increased toxicity when administered in combination. AZT alone, rifabutin alone, and combinations of the two compounds did not cause gross external alterations in pups. AZT alone caused decreases in pregnancy rates and live litter size and increased numbers of resorptions. Rifabutin alone caused decreased pregnancy rates and decreased mean fetal body weight per litter. Administration of AZT and

rifabutin in combinations caused marked decreases in pregnancy rates, live litter size, and mean fetal body weight per litter and marked increases in resorptions. 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 rifabutin combinations have the potential to markedly increase the hematopoietic toxicity of either compound alone.

REFERENCES

Amin, N.M. (1989). Zidovudine for treating AIDS: What physicians need to know. *Postgrad. Med.* **86**, 195-208.

Ayers, K.M. (1988). Preclinical toxicology of Zidovudine: An overview. *Am. J. Med.* **85**, 186-188.

Battaglia, R., Salgarollo, G., Zini, G., Montesanti, L., and Benedetti, M.S. (1991). Absorption, distribution, and urinary metabolism of ^{14}C-rifabutin in rats. *Antimicrob. Agents Chemother.* **35**, 1391-1396.

Chaisson, R.E., Moore, R.D., Richman, D.D., Keruly, J., and Creagh, T. (1992). Incidence and natural history of *Mycobacterium avium*-complex infections in patients with advanced human immunodeficiency virus disease treated with zidovudine. *Am. Rev. Respir. Dis.* **146**, 285-289.

Coffin, J.M. (1986). Genetic variation in AIDS viruses. *Cell* **46**, 1-4.

Dunn, O.J. (1964). Multiple comparisons using rank sums. *Technometrics* **6**, 241-252.

Dunnett, C.W. (1955). A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* **50**, 1095-1121.

Freireich, E.J., Gehan, E.A., Rall, D.P., Schmidt, L.H., and Skipper, H.E. (1966). Quantitative comparison of toxicity of anticancer agents in mouse, rat, hamster, dog, monkey, and man. *Cancer Chemother. Rep.* **50**, 219-244.

Gottlieb, M.S., Schroff, R., Schanker, H.M., Weisman, J.D., Fan, P.T., Wolf, R.A., and Saxon, A. (1981). *Pneumocystis carinii* pneumonia and mucosal candidiasis in previously healthy homosexual men: Evidence of a new acquired cellular immunodeficiency. *N. Engl. J. Med.* **305**, 1425-1431.

Greene, J.A., Ayers, K.M., deMiranda, P., and Tucker, W.E. (1990). Postnatal survival in Wistar rats following oral dosage with zidovudine on gestation day 10. *Fundam. Appl. Toxicol.* **15**, 201-206.

Harada, T., Enomoto, A., Boorman, G.A., and Maronpot, R.R. (1999). Liver and gallbladder. In *Pathology of the Mouse* (R.R. Maronpot, Ed.), pp. 129-130. Cache River Press, Vienna, IL.

Harris, M.W., Chapin, R.E., Lockhart, A.C., and Jokinen, M.P. (1992). Assessment of short-term reproductive and developmental toxicity screen. *Fundam. Appl. Toxicol.* **19**, 186-196.

Herzlich, B.C., Ranginwala, M., Nawabi, I., and Herbert, V. (1990). Synergy of inhibition of DNA synthesis in human bone marrow by azidothymidine plus deficiency of folate and/or vitamin B_{12}? *Am. J. Hematol.* **33**, 177-183.

Jain, N.C. (1986). *Schalm's Veterinary Hematology*. 4th ed., p. 549. Lea and Febiger, Philadelphia.

Jeffries, D.J. (1989). Targets for antiviral therapy of human immunodeficiency virus infection. *J. Infect.* **18** (Suppl. 1), 5-13.

Jonckheere, A.R. (1954). A distribution-free *k*-sample test against ordered alternatives. *Biometrika* **41**, 133-145.

Mansuri, M.M., Hitchcock, M.J., Buroker, R.A., Bregman, C.L., Ghazzouli, I., Desiderio, J.V., Starrett, J.E., Sterzycki, R.Z., and Martin, J.C. (1990). Comparison of *in vitro* biological properties and mouse toxicities of three thymidine analogs active against human immunodeficiency virus. *Antimicrob. Agents Chemother.* **34**, 637-641.

Masur, H. (1993). Recommendations on prophylaxis and therapy for disseminated *Mycobacterium avium* complex disease in patients infected with the human immunodeficiency virus. *N. Engl. J. Med.* **329**, 898-904.

Masur, H., Michelis, M A., Greene, J.B., Onorato, I., Stouwe, R.A., Holzman, R.S., Wormser, G., Brettman, L., Lange, M., Murray, H.W., and Cunningham-Rundles, S. (1981). An outbreak of community-acquired *Pneumocystis carinii* pneumonia: Initial manifestations of cellular immune dysfunction. *J. Immunol.* **135**, 3151-3162.

National Toxicology Program (NTP) (1999). Toxicology and Carcinogenesis Studies of AZT (CAS No. 30516-87-1) and AZT/α-Interferon A/D in $B6C3F_1$ Mice (Gavage Studies). Technical Report Series No. 469. NIH Publication No. 99-3959. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

Nightingale, S.D., Cameron, D.W., Gordin, F.M., Sullam, P.M., Cohn, D.L., Chaisson, R.E., Eron, L.J., Sparti, P.D., Bihari, B., Kaufman, D.L., Stern, J.J., Pearce, D.D., Weinberg, W.G., LaMarca, A., and Siegal, F.P. (1993). Two controlled trials of rifabutin prophylaxis against *Mycobacterium avium* complex infection in AIDS. *N. Engl. J. Med.* **329**, 828-833.

O'Brien, R.J., Lyle, M.A., and Snider, D.E., Jr. (1987). Rifabutin (Ansamycin LM 427): A new rifamycin-S derivative for treatment of mycobacterial diseases. *Rev. Infect. Dis.* **9**, 519-530.

Orme, I.M. (1988). Antimycobacterial activity *in vivo* of LM427 (rifabutin). *Am. Rev. Respir. Dis.* **138**, 1254-1257.

Physician's Desk Reference (PDR) (1995). 49th ed., pp. 1893-1895. Medical Economics Data, Medical Economics Company Inc., Montvale, N.J.

Richman, D.D. (1988). The treatment of HIV infection. Azidothymidine (AZT) and other new antiviral drugs. *Infec. Dis. Clin. North Am.* **2**, 397-497.

Saito, H., Sato, K., and Tomioka, H. (1988). Comparative *in vitro* and *in vivo* activity of rifabutin and rifampicin against *Mycobacterium avium* complex. *Tubercle* **69**, 187-192.

Scampini, G., Nava, A., Newman, A.J., Della Torre, P., and Mazue, G. (1993). Multinucleated hepatocytes induced by rifabutin in rats. *Toxicol. Pathol.* **21**, 369-376.

Scheffe, H. (1953). A method for judging all contrasts in the analysis of variance. *Biometrika* **40**, 87-104.

Siegal, F.P., Lopez, C., Hammer, C.S., Brown, A.E., Kornfeld, S.J., Gold., J., Hassett, J., Hirschman, S.Z., Cunningham-Rundles, C., Adelsburg, B.R., Parham, D.M., Siegal, M., Cunningham-Rundles, S., and Armstrong, D. (1981). Severe acquired immunodeficiency in male homosexuals manifested by chronic perianal ulcerative herpes simplex lesions. *N. Engl. J. Med.* **305**, 1439-1444.

Siegal, F.P., Eilbott, D.J., Burger, H., Gehan, K., Davidson, B., Kaell, A.T., and Weiser, B. (1990). Dose-limiting toxicity of rifabutin in AIDS-related complex: Syndrome of arthralgia/arthritis. *AIDS* **4**, 433-441.

Siegel, S. (1956). *Nonparametric Statistics for the Behavioral Sciences*, pp. 96-104. McGraw-Hill, New York.

Skinner, M.H., Hsieh, M., Torseth, J., Pauloin, D., Bhatia, G., Harkonen, S., Merigan, T.C., and Blaschke, T.F. (1989). Pharmacokinetics of rifabutin. *Antimicrob. Agents Chemother.* **33**, 1237-1241.

Snedecor, G.W., and Cochran, W.G., Eds. (1967a). Analysis of variance. In *Statistical Methods*, 6th ed., pp. 258-275. Iowa State University Press, Ames, IA.

Snedecor, G.W., and Cochran, W.G., Eds. (1967b). Analysis of covariance. In *Statistical Methods,* 6th ed., pp. 419-431. Iowa State University Press, Ames, IA.

Snedecor, G.W., and Cochran, W.G., Eds. (1967c). Test for a linear trend in proportions. In *Statistical Methods*, 6th ed., pp. 246-248. Iowa State University Press, Ames, IA.

Snower, D.P., and Weil, S.C. (1993). Changing etiology of macrocytosis. Zidovudine as a frequent causative factor. *Am. J. Clin. Pathol.* **99**, 57-60.

Sokal, R.R., and Rohlf, F.J. (1969). Kruskal-Wallis Test. In *Biometry*, pp. 370-371, 388-389. W.H. Freeman and Co., San Francisco.

Toltzis, P., Marx, C.M., Kleinman, N., Levine, E.M., and Schmidt, E.V. (1991). Zidovudine-associated embryonic toxicity in mice. *J. Infect. Dis.* **163**, 1212-1218.

Trang, J.M., Prejean, J.D., James, R.H., Irwin, R.D., Goehl, T.J., and Page, J.G. (1993). Zidovudine bioavailability and linear pharmacokinetics in female B6C3F1 mice. *Drug Metab. Dispos.* **21**, 189-193.

Vince, R., Hua, M., Brownell, J., Daluge, S., Lee, F.C., Shannon, W.M., Lavelle, G.C., Qualls, J., Weislow, O.S., Kiser, R., Canonico, C.G., Schultz, R.H., Narayanan, V.L., Mayo, L.G., Shoemaker, R.H., and Boyd, M.R. (1988). Potent and selective activity of a new carbocyclic nucleoside analog (Carbovir: NSC 614846) against human immunodeficiency virus *in vitro*. *Biochem. Biophys. Res. Commun.* **156**, 1046-1053.

Volpe, D.A., Du, D.L., Verhoef, V., and Murphy, M.J. (1993). Myelotoxicity of rifabutin and 3′-azido-3′-deoxythymidine, alone and in combination, to human hematopoietic progenitor cells *in vitro*. *Pathobiology* **61**, 77-82.

Wilcoxon, F. (1945). Individual comparison by ranking methods. *Biometrics* **1**, 80-83.

Williams, D.A. (1971). A test for differences between treatment means when several dose levels are compared with a zero dose control. *Biometrics* **27**, 103-117.

Williams, D.A. (1972). The comparison of several dose levels with a zero dose control. *Biometrics* **28**, 519-531.

APPENDIX A
CLINICAL PATHOLOGY RESULTS

TABLE A1
Hematology Data for Male Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations[a]

Dose	WBC[b] (10^3/mm^3)	RBC (10^6/mm^3)	Hgb (g/dL)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	Platelets (10^3/mm^3)
0 + 0	6.97 1.960	9.47 0.536	15.9 0.86	45.7 2.00	48.3 1.76	16.8 0.53	34.9 0.96	992 266.3
200 + 0	5.37 1.786	8.36 0.550	14.7 0.88	43.1 2.90	51.6 2.66	17.6 0.67	34.1 1.22	1,320 127.9
400 + 0	4.81 1.501	8.33* 0.556	14.6 0.76	43.1 3.02	51.7 2.28	17.6 0.71	34.1 0.99	1,469 402.6
0 + 80	5.62 2.289	9.51 0.692	15.7 0.94	46.2 3.44	48.6 1.66	16.5 0.47	34.0 0.99	1,088 243.6
200 + 80	5.83 1.613	8.15* 0.533	14.2 1.01	41.8 2.46	51.3 1.93	17.5 0.85	34.1 0.75	1,439 407.3
400 + 80	6.14 2.424	7.43** 0.561	13.2** 0.71	38.4* 2.03	51.8 2.18	17.8 0.76	34.4 0.97	1,693** 442.8
0 + 320[c]	8.24 2.042	8.47 0.617	13.7* 1.11	40.7 2.23	48.2 1.74	16.2 0.55	33.7 1.24	1,300 302.9
200 + 320	6.49 2.169	6.99** 0.521	12.5** 0.77	38.1* 2.48	54.6** 1.95	17.9* 0.60	32.8 0.45	1,497* 275.7
400 + 320[c]	5.04 1.206	6.09** 1.488	10.5** 2.48	32.0** 8.40	52.2 5.83	17.3 1.23	33.3 2.08	1,562* 650.3
0 + 640[c]	8.26 2.971	8.65 0.879	13.6* 1.12	39.7 4.00	45.9 0.91	15.7 0.56	34.2 1.12	1,357 116.4
200 + 640	6.02 3.425	4.13** 2.012	7.3** 3.92	21.9** 13.98	49.8 10.92	17.3 1.82	35.6 4.92	1,921** 410.1
400 + 640[c]	2.92 1.246	2.31** 0.458	3.7** 0.73	9.2** 2.08	39.7** 3.55	15.9 0.63	40.1** 2.56	2,135** 825.1

TABLE A1
Hematology Data for Male Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations

Dose	Reticulocytes (10^5/mm^3)	Neutrophils (10^3/mm^3)	Lymphocytes (10^3/mm^3)	Monocytes (10^3/mm^3)	Eosinophils (10^3/mm^3)	Basophils (10^3/mm^3)	LUC[d] (10^3/mm^3)
0 + 0	4.5	1.00	5.53	0.13	0.24	0.02	0.05
	0.64	0.304	1.673	0.053	0.110	0.011	0.021
200 + 0	5.0	0.87	4.24	0.08	0.15	0.01	0.03[e]
	0.90	0.342	1.591	0.074	0.051	0.010	0.033
400 + 0	4.9	0.68	3.82	0.10	0.18	0.01	0.03[f]
	1.02	0.235	1.286	0.054	0.091	0.005	0.014
0 + 80	5.3	0.93	4.32	0.09	0.23	0.02	0.03
	0.64	0.368	1.849	0.040	0.149	0.011	0.020
200 + 80	4.9	0.75	4.66	0.10	0.25	0.01	0.06
	0.63	0.229	1.524	0.035	0.091	0.007	0.033
400 + 80	5.1	0.68	5.08	0.11	0.22	0.02	0.05[f]
	0.78	0.290	2.002	0.060	0.198	0.013	0.030
0 + 320[c]	6.6	1.75	6.04	0.14	0.21	0.03	0.08
	2.33	0.524	1.655	0.042	0.106	0.011	0.038
200 + 320	6.1	1.16	4.92	0.10	0.23	0.02	0.07
	1.46	0.513	1.679	0.042	0.082	0.013	0.043
400 + 320[c]	7.3	0.93	3.77	0.08	0.18	0.01	0.07
	4.01	0.667	0.748	0.049	0.157	0.010	0.032
0 + 640[c]	8.1	2.04**	5.73	0.13	0.24	0.03	0.09
	0.91	0.599	2.638	0.041	0.192	0.020	0.064
200 + 640	6.3	1.49	3.95	0.13	0.26	0.01	0.19**[f]
	6.74	1.432	2.040	0.082	0.393	0.015	0.169
400 + 640[c]	1.1	0.74	2.05	0.04	0.04	0.00	0.06
	0.50	0.642	0.645	0.039	0.032	0.005	0.042[g]

* Significantly different ($P \leq 0.05$) from the vehicle control group using analysis of variance followed by Dunnett's test
** $P \leq 0.01$
[a] Daily gavage doses of AZT + rifabutin (mg/kg per day). For each parameter, the mean is presented above the standard deviation; n=10, unless otherwise noted.
[b] WBC counts corrected for RBC test counts greater than 10 per 100 WBCs
[c] n=7
[d] Large unstained cells
[e] n=8
[f] n=9
[g] n=6

TABLE A2
Hematology Data for Female-A Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations[a]

Dose	WBC[b] (10^3/mm^3)	RBC (10^6/mm^3)	Hgb (g/dL)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	Platelets (10^3/mm^3)
0 + 0	7.68 2.671	8.74 0.624	14.6 0.92	42.8 2.52	49.1 1.88	16.8 0.73	34.1 1.04	1,382 339.7
200 + 0	5.69* 1.548	7.51** 0.594	14.0 0.73	41.9 2.62	55.9** 3.06	18.8** 1.00	33.6 0.85	1,672 206.8
400 + 0	5.86 1.111	7.71** 0.710	14.6 1.00	43.7 3.12	56.8** 3.17	19.0** 0.95	33.4 0.97	1,379 474.3
0 + 80[c]	8.28 3.034	8.81 0.648	14.7 0.91	43.5 2.62	49.4 1.84	16.8 0.71	33.9 1.17	1,163 416.5
200 + 80	6.34 1.934	7.11** 0.785	13.5 1.37	40.7 4.26	57.3** 2.07	19.0** 0.79	33.2 1.01	1,509 233.6
400 + 80[d]	5.32* 1.470	6.96** 0.702	13.2 1.16	39.7 3.89	57.1** 2.06	19.0** 0.54	33.3 0.79	1,354 335.2
0 + 320[c]	8.65 2.542	8.88 0.463	14.5 0.88	44.0 2.48	49.6 1.96	16.3 0.82	32.9 0.80	1,217 232.1
200 + 320[e]	6.41 2.879	5.99** 2.017	11.7** 4.01	35.6** 12.47	58.0** 8.74	19.2** 1.83	33.4 2.65	1,504 322.5
400 + 320[f]	4.34 2.566	2.85** 2.633	5.2** 4.97	15.8** 17.3	49.6 10.52	17.6 0.95	36.3 5.50	2,279 683.4
0 + 640[c]	11.18** 2.250	9.23 0.885	14.6 1.15	43.3 4.18	47.0 2.05	15.9 0.49	33.8 1.66	1,218 314.3
200 + 640[g]	—	—	—	—	—	—	—	—
400 + 640[g]	—	—	—	—	—	—	—	—

TABLE A2
Hematology Data for Female-A Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations

Dose	Reticulocytes (10^5/mm^3)	Neutrophils (10^3/mm^3)	Lymphocytes (10^3/mm^3)	Monocytes (10^3/mm^3)	Eosinophils (10^3/mm^3)	Basophils (10^3/mm^3)	LUC[h] (10^3/mm^3)
0 + 0	4.1 0.96	2.62 1.584	4.67 1.137	0.17 0.069	0.15 0.081	0.02 0.013	0.05 0.054
200 + 0	4.2 0.89	1.10** 0.444	4.21 1.272	0.11 0.054	0.22 0.161	0.01 0.007	0.03 0.014
400 + 0	4.6 1.56	1.19** 0.600	4.31 0.897	0.14 0.083	0.16 0.092	0.01 0.007	0.04[i] 0.029
0 + 80[c]	4.6 1.49	2.65 2.058	5.10 1.014	0.20 0.089	0.23 0.217	0.02 0.018	0.07[j] 0.114
200 + 80	4.3 1.29	1.19** 0.600	4.78 1.398	0.12 0.058	0.20 0.140	0.02 0.008	0.04 0.019
400 + 80[d]	5.4 2.11	1.07** 0.771	3.90 0.975	0.09 0.051	0.21 0.116	0.01 0.009	0.04[j] 0.022
0 + 320[c]	3.9 1.09	2.83 1.224	5.43 1.837	0.13 0.065	0.16 0.134	0.03 0.015	0.08 0.046
200 + 320[e]	3.7[d] 1.67	1.66 0.995	4.29 1.890	0.11 0.060	0.25 0.163	0.01 0.011	0.08 0.053
400 + 320[f]	2.4 2.53	0.91 0.692	3.22 1.636	0.09 0.107	0.10 0.153	0.00 0.006	0.02[k] 0.007
0 + 640[c]	5.7 2.08	3.85* 1.818	6.70 1.606	0.18 0.080	0.16 0.066	0.07 0.102	0.25** 0.218
200 + 640[g]	—	—	—	—	—	—	—
400 + 640[g]	—	—	—	—	—	—	—

* Significantly different ($P \leq 0.05$) from the vehicle control group using analysis of variance followed by Dunnett's test
** $P \leq 0.01$
[a] Daily gavage doses of AZT + rifabutin (mg/kg per day). For each parameter, the mean is presented above the standard deviation; n=20, unless otherwise noted.
[b] WBC counts corrected for RBC test counts greater than 10 per 100 WBCs
[c] n=12
[d] n=14
[e] n=15
[f] n=3
[g] No data were available due to 100% mortality in this group.
[h] Large unstained cells
[i] n=19
[j] n=11
[k] n=2

TABLE A3
Hematology Data for Female-B Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations[a]

Dose	WBC[b] (10^3/mm^3)	RBC (10^6/mm^3)	Hgb (g/dL)	Hct (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	Platelets (10^3/mm^3)
0 + 0[c]	4.92	8.44	14.5	43.4	51.4	17.2	33.4	1,262
	1.452	0.542	0.81	2.18	1.70	0.49	0.64	279.3
200 + 0[c]	5.85	7.91	14.0	42.4	53.8	17.8	33.0	1,522
	1.577	0.538	0.58	1.43	2.76	1.02	0.95	259.9
400 + 0[d]	5.44	8.28	14.5	44.1	53.3	17.5	32.9	1,262
	1.838	0.614	0.98	2.70	2.79	0.89	0.99	276.2
0 + 80[c]	5.46	8.83	14.7	43.6	49.7	16.7	33.7	1,358
	1.376	0.913	1.10	2.64	3.10	0.78	1.29	258.2
200 + 80[c]	5.66	8.05	14.3	43.3	54.0	17.8	33.0	1,267
	1.506	0.758	0.98	3.38	2.83	0.73	1.09	343.8
400 + 80	5.55	8.31	14.8	44.2	53.4	17.9	33.5	1,242
	1.550	0.671	0.83	2.08	3.14	0.93	0.66	266.3
0 + 320[e]	6.45	8.66	14.5	44.3	51.2	16.8	32.8	1,455
	1.793	0.697	1.17	3.29	2.58	0.71	0.96	286.2
200 + 320	6.06	7.98	14.2	43.6	54.7**	17.8	32.5	1,352
	0.985	0.514	0.70	2.30	2.72	0.75	0.63	268.5
400 + 320[f]	6.71	8.35	14.5	45.4	54.4*	17.4	32.0**	1,545
	1.853	0.744	1.40	4.37	2.77	0.71	0.94	551.9
0 + 640	5.44	8.37	13.8	42.1	50.3	16.5	32.9	1,644
	1.645	0.727	1.13	3.56	1.53	0.65	1.00	485.9
200 + 640	6.40	7.63*	13.6	42.9	56.6**	17.8	31.6**	1,608
	2.859	1.025	1.81	4.66	4.06	0.79	1.79	488.3
400 + 640	8.46	7.33**	12.9	42.1	57.4**	17.6	30.7**	1,786
	5.618	0.982	1.90	5.81	1.85	0.53	1.09	608.4

TABLE A3
Hematology Data for Female-B Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations

Dose	Reticulocytes (10^5/mm^3)	Neutrophils (10^3/mm^3)	Lymphocytes (10^3/mm^3)	Monocytes (10^3/mm^3)	Eosinophils (10^3/mm^3)	Basophils (10^3/mm^3)	LUC[g] (10^3/mm^3)
0 + 0[c]	4.6	1.06	3.54	0.10	0.20	0.01	0.02[h]
	1.27	0.328	1.188	0.031	0.212	0.011	0.013
200 + 0[c]	5.5	1.07	4.48	0.10	0.15	0.02	0.03
	1.75	0.305	1.338	0.040	0.095	0.010	0.011
400 + 0[d]	6.1	1.02	4.11	0.10	0.18	0.01	0.02[i]
	2.46	0.481	1.530	0.051	0.088	0.008	0.015
0 + 80[c]	4.5	1.12	4.07	0.10	0.14	0.01	0.02[j]
	1.67	0.595	1.054	0.069	0.121	0.010	0.009
200 + 80[c]	4.8	0.93	4.36	0.09	0.24	0.01	0.03[h]
	1.47	0.273	1.285	0.046	0.156	0.011	0.012
400 + 80	5.5	1.09	4.06	0.11	0.26	0.01	0.03[c]
	2.05	0.385	1.130	0.048	0.167	0.007	0.016
0 + 320[e]	5.4	1.19	4.92	0.12	0.19	0.02	0.03[k]
	1.78	0.525	1.387	0.087	0.171	0.012	0.012
200 + 320	5.7	1.18	4.51	0.11	0.22	0.02	0.03[c]
	2.43	0.425	0.964	0.030	0.114	0.008	0.017
400 + 320[f]	7.4	1.30	4.95	0.12	0.27	0.02	0.05
	6.16	0.391	1.469	0.053	0.225	0.014	0.056
0 + 640	7.0	1.24	3.84	0.12	0.18	0.01	0.04[c]
	1.98	0.383	1.256	0.083	0.108	0.010	0.026
200 + 640	7.3	1.43	4.53	0.13	0.26	0.02	0.03[c]
	4.73	0.698	2.215	0.065	0.210	0.011	0.024
400 + 640	9.0	2.27	5.63	0.17	0.31	0.02	0.07[j]
	5.11	2.247	3.529	0.087	0.148	0.019	0.058

* Significantly different ($P \leq 0.05$) from the vehicle control group using analysis of variance followed by Dunnett's test
** $P \leq 0.01$
[a] Daily gavage doses of AZT + rifabutin (mg/kg per day). For each parameter, the mean is presented above the standard deviation; n=16, unless otherwise noted.
[b] WBC counts corrected for RBC test counts greater than 10 per 100 WBCs
[c] n=15
[d] n=20
[e] n=17
[f] n=12
[g] Large unstained cells
[h] n=13
[i] n=18
[j] n=14
[k] n=16

TABLE A4
Clinical Chemistry Data for Male Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations[a]

Dose	ALP (IU/L)	ALT (IU/L)	AST (IU/L)	SDH (IU/L)	Bile Acids (μmol/L)
0 + 0[b]	56	26	71[e]	20[e]	15[f]
	30.4	7.0	22.7	4.4	10.7
200 + 0[c]	55	23	59[g]	23[e]	7[h]
	16.1	5.2	33.6	4.9	1.4
400 + 0[c]	69	29	71	24[g]	13[e]
	20.3	4.9	22.7	5.9	9.2
0 + 80[c]	53	24	64[e]	22[i]	12[i]
	11.6	4.5	13.9	7.7	8.4
200 + 80[b]	59	25	54[e]	19[e]	12[f]
	11.5	5.6	6.3	9.7	7.4
400 + 80[c]	52	24	55[b]	27[g]	18[f]
	12.6	6.6	20.7	8.0	18.2
0 + 320	43	35	80	23[e]	19[i]
	10.3	10.9	31.1	5.6	16.1
200 + 320[d]	66	36	65	22[g]	17[e]
	22.8	29.2	17.1	8.6	13.3
400 + 320	43	41	72	30[e]	16[i]
	10.1	23.8	17.8	13.8	5.6
0 + 640	64	65	90[e]	17[i]	12[j]
	21.2	53.5	16.5	6.4	9.7
200 + 640[d]	63	31	74	27[g]	54**[e]
	13.1	19.5	21.6	6.4	37.9
400 + 640	67	21	57	25	48*[e]
	16.9	9.2	13.4	5.2	8.0

* Significantly different ($P \leq 0.05$) from the vehicle control group using analysis of variance followed by Dunnett's test
** $P \leq 0.01$
[a] Daily gavage doses of AZT + rifabutin (mg/kg per day). For each parameter, the mean is presented above the standard deviation; n=7, unless otherwise noted.
[b] n=8
[c] n=9
[d] n=10
[e] n=6
[f] n=4
[g] n=7
[h] n=2
[i] n=5
[j] n=3

TABLE A5
Clinical Chemistry Data for Female-A Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations[a]

Dose	ALP (IU/L)	ALT (IU/L)	AST (IU/L)	SDH (IU/L)	Bile Acids (μmol/L)
0 + 0	52 10.8	39 16.6	118 31.4	31[b] 6.6	15[h] 8.9
200 + 0[b]	71 21.4	27 7.5	87 37.7	26[i] 5.2	11[j] 6.3
400 + 0	74 20.9	31 12.1	99[k] 47.5	25[k] 5.5	12[e] 5.5
0 + 80[c]	40 8.5	30 5.8	94 16.7	23[l] 4.7	12[m] 3.3
200 + 80	52 11.1	25 6.8	87 23.1	27[k] 3.5	12[k] 6.4
400 + 80[d]	65 17.4	23 7.4	78 30.7	24[m] 7.6	15[l] 8.4
0 + 320[e]	47 11.8	75* 50.3	122 45.7	30[c] 9.4	19[c] 9.3
200 + 320[d]	63 25.4	97** 65.9	124[j] 78.2	36[n] 5.4	25[o] 11.2
400 + 320[f]	82 3.0	30 18.2	109 58.3	29[p] 1.4	60** 37.3
0 + 640[e]	67 24.6	159** 81.9	200 120.6	29[q] 10.3	31**[m] 10.8
200 + 640[g]	—	—	—	—	—
400 + 640[g]	—	—	—	—	—

* Significantly different ($P \leq 0.05$) from the vehicle control group using analysis of variance followed by Dunnett's test
** $P \leq 0.01$
[a] Daily gavage doses of AZT + rifabutin (mg/kg per day). For each parameter, the mean is presented above the standard deviation; n=20, unless otherwise noted.
[b] n=19
[c] n=11
[d] n=14
[e] n=12
[f] n=3
[g] No data were available due to 100% mortality in this group.
[h] n=15
[i] n=18
[j] n=13
[k] n=17
[l] n=10
[m] n=8
[n] n=7
[o] n=9
[p] n=2
[q] n=6

APPENDIX B
LIVER WEIGHTS

TABLE B1
Mean Absolute Liver Weights for Male and Female-A Swiss (CD-1®) Mice in the Reproductive, Developmental, and General Toxicity Study of AZT and Rifabutin Combinations[a]

Dose	Male	Female-A
0 + 0	1.7940 ± 0.2359	2.5388 ± 0.1378
0 + 80	1.8280 ± 0.1468	2.7959 ± 0.1668[b]
0 + 320	1.9620 ± 0.1526	2.6660 ± 0.5721[c]
0 + 640	2.2410 ± 0.2725*	3.4033 ± 0.2499*[d]
200 + 0	1.7590 ± 0.1642	2.4057 ± 0.4349[e]
200 + 80	1.7970 ± 0.1215	2.4692 ± 0.3619[f]
200 + 320	2.0920 ± 0.3185*	2.7300 ± 0.8778[b]
200 + 640	2.1690 ± 0.2907*	—[g]
400 + 0	1.7800 ± 0.1658	2.2706 ± 0.4702
400 + 80	1.9090 ± 0.2037	2.1150 ± 0.3142[h]
400 + 320	2.0130 ± 0.2193*	1.470[i]
400 + 640	1.8580 ± 0.3247	—

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test

[a] Daily gavage doses of AZT + rifabutin (mg/kg per day). Mean absolute liver weights given in grams (mean ± standard deviation); for males, n=10; for females, n=16, unless otherwise noted.

[b] n=9

[c] n=10

[d] n=3

[e] n=14

[f] n=13

[g] No data were available due to 100% mortality in this group.

[h] n=4

[i] n=1

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