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BMS-189453, a Novel Retinoid Receptor Antagonist, Is a Potent Testicular Toxin

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BMS-189453 is a synthetic retinoid that acts as an antagonist at retinoic acid receptors α, β, and γ. In Sprague Dawley rats at daily oral doses of 15, 60, or 240 mg/kg for 1 month, BMS-189453 produced increases in leukocyte counts, alkaline phosphatase and alanine aminotransferase levels, and marked testicular degeneration and atrophy at all doses. Significant overt signs of toxicity and deaths occurred at 240 mg/kg, whereas body-weight and food-consumption decreases occurred at 60 and 240 mg/kg. When BMS-189453 was administered to male rats at daily doses ranging from 12.5 to 100 mg/kg for 1 week, only minimal testicular changes occurred at all doses, shortly after the dosing period. However, after a 1-month drug-free observation period, marked testicular atrophy was evident at all doses. BMS-189453 was then administered at doses of 2, 10, or 50 mg/kg to male rats for 1, 3, or 7 consecutive days. Dose- and duration-dependent testicular toxicity that occurred after a 1-month observation period did not recover, and, in some cases, was more severe 4 months after the last dose. In rabbits administered BMS-189453 at oral doses of 2, 10, or 50 mg/kg for 1 week, testicular degeneration and atrophy were evident in the high-dose group at 1 month following treatment. These studies indicate that retinoid antagonists can selectively produce progressive and prolonged testicular toxicity after single or repeated oral doses that are otherwise well tolerated.

Key Words: **retinoic acid receptor antagonist; testicular toxicity; rats; rabbits; toxicity; retinoids; Vitamin A; oral; *in vivo*; safety assessment.**

Retinoids are a class of compounds consisting of natural and synthetic analogs of vitamin A. Vitamin A regulates growth and differentiation in many cell types of endodermal, mesodermal, and ectodermal origin and is required for normal growth, bone formation, vision, reproduction, and epithelial differentiation (Lotan, 1980; Moore, 1967). Retinoids exert their effects on morphogenesis, differentiation, and cellular proliferation through interactions with specific nuclear retinoic-acid receptors (RAR) or retinoid X receptors (RXRs). At least 3 specific RAR subtypes have been identified and designated as RAR α, β, and γ (Mangelsdorf *et al*., 1984). These receptors are members of the thyroid/steroid super-family of receptors. RARs are thought to function *in vivo* as heterodimers in combination with RXRs. RXRs also form heterodimeric partners with thyroid receptors, peroxisome proliferator-activated receptors, and vitamin-D receptors, thereby extending their potential biological functions (Mangelsdorf *et al*., 1994).

Synthetic vitamin A analogs are used successfully in the treatment of skin diseases such as acne, psoriasis, and other keratinizing dermatoses (Orfanos *et al*., 1987, 1997). They are also being investigated for potential activity as anticancer agents and in cancer chemoprevention (Armstrong *et al*., 1992; Matsushimi *et al*., 1992; Meister *et al*., 1998). Therapeutic use of these compounds is limited by a number of side effects, including mucocutaneous toxicity (cheilitis, xerosis, epistaxis, pruritus, desquamation, erythema, and scaling), headache, hypertriglyceridemia, teratogenicity, and bone toxicity (Armstrong *et al*., 1992; Kamm, 1982; Kovacs and Shear, 1993). Retinoid toxicities are mediated through activation of the RARs (Standeven *et al*., 1996a,b,c; Thatcher *et al*., 1997), and may be blocked or retarded by the actions of RAR antagonists (Kochhar *et al*., 1998; Standeven *et al*., 1996a,b,c). A number of retinoid antagonists have been synthesized (Apfel *et al*., 1992; Eyrolles *et al*., 1994) with varying degrees of receptor subtype specificity. Retinoid-receptor antagonists have proven to be useful mechanistic tools for delineating the contribution of retinoid receptor subtypes in mediating the biological activity of retinoids (Yang *et al*., 1999). These antagonists have been shown to block various retinoid-induced toxicities including hyperlipidemia, teratogenicity, and bone toxicity, and have shown preclinical utility as a treatment for retinoid intoxication (Apfel *et al*., 1992; Eckhardt and Schmitt, 1994; Standeven *et al*., 1996, 1997).

BMS-189453 is a synthetic RAR antagonist having good (82–98%) oral bioavailability in rats and monkeys. BMS-189453 binds but does not activate the α, β, and γ retinoid receptors (Gehin *et al*., 1999). BMS-189453 is chemically classified as an "arotinoid" (Loeliger *et al*., 1980). The chemical structure of BMS-189453 is shown in Figure 1.

The therapeutic usefulness of RAR antagonists in the treatment of retinoid toxicity is self-evident. In addition, RAR

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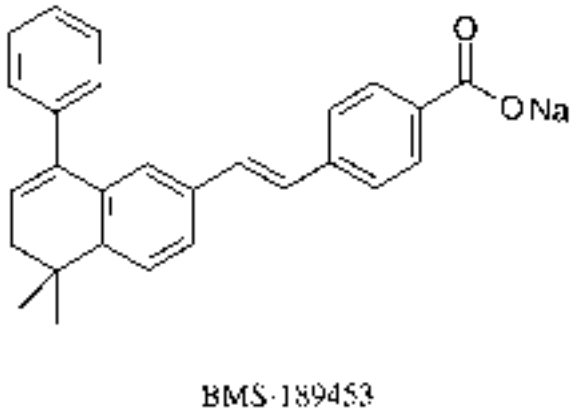

FIG. 1. The chemical structure of BMS-189453.

antagonists may be useful in treating dermatologic and inflammatory diseases because of the pronounced influence of retinoid-receptor stimulation on collagen synthesis and IL-8 production (Bollag, 1985; Olsen *et al*., 1990; Vincenti *et al*., 1994; Wang and Guda, 1988). Although a large body of information exists regarding the toxicity of retinoid agonists (Hixson *et al*., 1979; Kamm, 1982; Kistler *et al*., 1990; Kurtz *et al*., 1984; Lindamood *et al*., 1987, 1990), the toxicity of retinoid antagonists utilizing *in vivo* experimental models has not been extensively studied. Therefore, studies were performed to investigate the potential target-organ toxicity of BMS-189453 in rats following 1 month of oral administration. Additionally, 1-week and 1-, 3-, or 7-day oral toxicity studies were conducted in rats, and a 1-week oral toxicity study was conducted in rabbits, to investigate the dose-response relationships, effects of dosing duration, species selectivity, and reversibility of testicular toxicity that were noted in the 1-month rat study.

MATERIALS AND METHODS

Drugs. BMS-189453 (Batch Number N005A), supplied by Bristol-Myers Squibb Pharmaceutical Research Institute (Buffalo, NY) was used as the sodium salt. For oral administration, BMS-189453 was suspended in a vehicle of aqueous 1.5% microcrystalline cellulose and carboxymethylcellulose (Avicel®, Dow Chemical, Midland, MI) to achieve the desired concentrations. All doses were administered in terms of free acid. Dosing suspensions were prepared fresh daily and administered immediately following preparation.

Animals. Sprague-Dawley rats (males 8 or 11 weeks of age, 206–290 g; females 8 weeks of age, 154–187 g) were received from Harlan Sprague Dawley, Frederick, MD and individually housed in stainless-steel cages with Purina® Certified Rodent Chow (#5002) and water available *ad libitum*. In the animal room, a 12-h light/dark cycle (lights on at 0600 h), a temperature range of 73 ± 5° F, and a relative humidity range of 45 ± 15% were maintained. After a 2-week acclimation period, rats were randomly assigned to groups, via a computer-generated random list, and identified by tail tattoos.

Male New Zealand White rabbits (approximately 1 year of age, 2–4 kg) were received from Hazleton Research Products, Denver, PA and individually housed in stainless-steel cages and fed Certified Rabbit Diet (#5325, PMI Feeds, Inc., St. Louis, MO). In the animal room, a 12-h light/dark cycle (lights on at 0600), a temperature range of 67 ± 6°F, and a relative humidity range of 45 ± 15% were maintained. After a 1-month acclimation period, rabbits were randomly assigned to groups, via a computer-generated random list, and identified by ear tags. All animal use was approved by the Institutional Animal Care and Use Committee and was in accordance with the USDA guidelines for humane animal care.

Experimental designs. For each experiment described below, BMS-189453 was administered by gavage once daily at a dose volume of 10 ml/kg. All animals were observed at least once daily for changes in condition and behavior, body weights were measured twice weekly, food consumption was measured once weekly (rats) or daily (rabbits), and physical examinations were conducted pretreatment and prior to scheduled necropsies. At the scheduled necropsies, all animals were euthanized by pentobarbital anesthesia followed by exsanguination. At necropsy, all major organs and tissues were examined for gross visible lesions, selected organs were weighed, and all major tissues were fixed and preserved in 10% formalin (Experiments 1 and 4 below) except the testes and right epididymis, which were preserved in Bouin's fixative (in all experiments). Subsequently, tissues were embedded in paraffin, sectioned at 2–4 μm, and stained with periodic acid Schiff (PAS) and/or hematoxylin and eosin. For Experiments 1 and 4, all tissues from the high-dose and control groups and target organs from all groups were examined microscopically, whereas for Experiments 2 and 3, the testes and epididymides from all groups were examined. For experiments where epididymal sperm counts were performed (i.e., Experiments 1 and 2 below), one epididymis (left) was separated from the testis, trimmed, weighed, and immediately frozen. The epididymal sperm counts were then determined as described by Blazak *et al*., 1985.

Experiment 1: One-month oral toxicity study in rats. BMS-189453 was administered orally via gavage to male and female rats (10/sex) at 16, 60, or 240 mg/kg for 30 days. Controls received 10 ml/kg/day of aqueous 1.5% Avicel®. Blood and urine samples were collected following an overnight fast during week 4, for routine clinical pathology evaluation.[2] All rats were sacrificed on day 30 and subjected to a gross necropsy. Selected organ weights and all major tissues were collected, preserved, processed, and examined microscopically as described above. Epididymal sperm counts were conduced as described above.

Doses for this study were chosen based on results from a preliminary 2-week pharmacology study in rats, in which edema of paws and swelling and redness of the ears occurred at 75 mg/kg/day. These effects were reduced when the dose was lowered to 50 mg/kg/day. Based on these results, doses of 15, 60, and 240 mg/kg were chosen for study. The high dose was expected to cause significant toxicity, the intermediate dose was expected to cause minimal toxicity, and the low dose was expected to cause little or no toxicity. In addition, the low dose of 60 mg/kg was 6-fold higher than the predicted human therapeutic dose of 10 mg/kg.

Experiment 2: One-week oral toxicity study in rats. BMS-189453 was administered orally via gavage to male rats (10/group) at daily doses of 12.5, 25, 50, or 100 mg/kg for 7 days. Controls received 10 ml/kg of aqueous 1.5% Avicel® for 7 days. The first 5 rats/group were sacrificed on day 8, and the remaining rats on day 36, and all were subjected to gross necropsy. The testis and epididymides from all animals were weighed, preserved, processed, and examined microscopically. In addition, epididymal sperm counts were conducted as described above.

Daily doses ranging from 12.5 to 100 mg/kg were selected, based on the

[2] Clinical pathology tests included hematology–erythrocyte count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, platelet count, reticulocyte count, and total and differential (absolute) leukocyte counts, prothrombin time, activated partial thromboplastin time, and fibrinogen. Serum chemistry included alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, glucose, urea nitrogen, creatinine, total protein, albumin, globulins, A/G ratio, cholesterol, triglycerides, total bilirubin, calcium, phosphorus, sodium, potassium, and chloride. Urinalysis tests included: volume, color, appearance, specific gravity, pH, microscopic examination of urinary sediment; and qualitative determination of protein, glucose, ketone, bilirubin, blood, and urobilinogen.

testicular toxicity observed in the previous 1-month oral toxicity study. A 1-week duration of dosing was chosen to determine the effect of a shorter dosing duration on the testicular toxicity observed in the previous study. In addition, a 1-month post-dose period was incorporated into the study design to assess the reversibility or progression of testicular changes.

Experiment 3: One-, three-, and seven-day oral toxicity study in rats. BMS-189453 was administered orally via gavage to male rats (10/group) at daily doses of 2, 10, or 50 mg/kg for 1, 3, or 7 days (9 separate groups). Controls received 10 ml/kg/day of aqueous 1.5% Avicel® for 7 days. The first 5 rats/group were sacrificed on day 35, and the remaining rats were sacrificed on day 124, and all were subjected to gross necropsy. The testis and epididymides from all animals were weighed, preserved, processed, and examined microscopically.

Doses were chosen based on the results of the previous 1-week oral toxicity study. Variable 1-, 3-, or 7-day dosing schedules and lower doses of 2, 10, or 50 mg/kg were chosen to determine the effect of shorter dosing duration and lower BMS-189453 doses on the testicular toxicity observed in the previous study. In addition, necropsies were scheduled at 1 and 4 months post-dose to assess the reversibility or progression of the testicular changes.

One-week oral toxicity study in rabbits. BMS-189453 was administered orally via gavage to male rabbits (5/group) at daily doses of 2, 10, or 50 mg/kg for 7 days. Controls received 10 ml/kg of aqueous 1.5% Avicel® for 7 days. The rabbit was chosen to assess interspecies differences in testicular toxicity following oral administration of BMS-189453. Doses of 2, 10, or 50 mg/kg were identical to those used in the previous rat study to allow comparison of equivalent doses between the species. Necropsies were conducted on the first 2 rabbits/group at the end of the 1-week dosing period (day 8) and on the remaining rabbits at 1 month post-dose (day 36) to assess progression or reversibility of testicular changes. Selected organs were weighed, all major tissues were collected, preserved, processed and examined microscopically as describe previously.

Data analysis. Body weight, absolute and relative (to body weight) organ weights, and clinical laboratory data in treated groups were compared to those of the control group by Dunnett's test (Dunnett, 1962). Statistical analysis of the anatomic pathology data was performed by Fisher's Exact test (Dixon and Massey, 1983). We considered p values < 0.05 to be statistically significant. The term significant, when used in describing results, refers to statistically significant differences unless stated otherwise.

RESULTS

One-Month Oral Toxicity Study in Rats

Drug-related deaths occurred in one male and one female at 240 mg/kg on days 20 and 18, respectively. One male at 15 mg/kg died on day 23 as the result of a gavage accident. Hair-coat soiling, salivation, sneezing, respiratory rales, and discharge from the nose, eyes and/or penis occurred transiently at all doses. Other clinical signs noted only at 60 and 240 mg/kg included transient dehydration, decreased activity, hunched-body posture, and red discoloration of the skin. Additional clinical findings at 240 mg/kg consisted of transient rough hair-coat; swelling of the ears, muzzle, and/or feet; body tremor; abdominal bloating; ptosis; and thin appearance. Of these observations, only body tremor was unique to the 2 rats that died.

Mean body weight and food consumption values were comparable to controls at 15 mg/kg (Fig. 2). In males at 60 mg/kg, mean body weight and food consumption values were significantly lower (12 to 15% and 11 to 29%, respectively) than

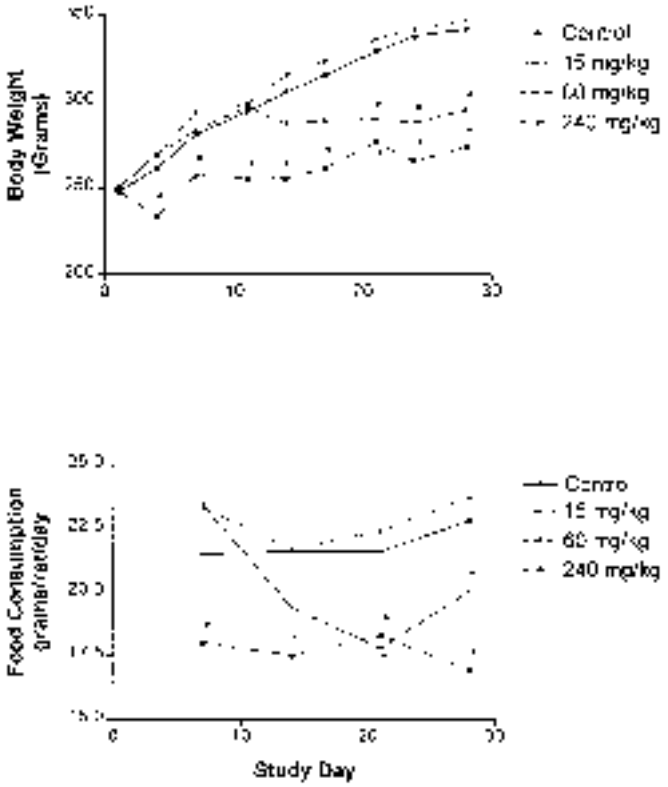

FIG. 2. Effects of 1-month administration of BMS-189453 on body weight and food consumption in male rats. Each point represents the mean; $n = 10$, except for the 15- and 240-mg/kg groups after days 23 and 20, respectively, where $n = 9$. Asterisks represent significant differences from control as determined by Dunnett's test ($p < 0.05$).

controls, beginning on day 22, and remained decreased for the study duration. However, at 240 mg/kg, mean body weight and food consumption values were significantly lower (9 to 22% and 15 to 26%, respectively) than controls for the study duration. Decreases in mean body weight and food consumption values also occurred in females at 240 mg/kg, but were not statistically significant (data not shown).

BMS-189453-related changes in neutrophil counts, serum alkaline phosphatase (ALK PHOS), and serum alanine aminotransferase (ALT) were noted during week 4 (Table 1). In males, significant dose-related increases (2.5- to 3-fold) in neutrophil counts were noted at 60 and 240 mg/kg. Two to 5-fold increases occurred in females but were not statistically significant. Significant dose-related increases (1.7- to 4.8-fold) in ALK PHOS and ALT occurred at all doses in both sexes, with the exception of the mean ALT value in males at 15 mg/kg, which was not statistically significant.

At necropsy, testicular weights and epididymal sperm counts were significantly decreased (57 to 63% and 91 to 93%, respectively) at all doses (Fig. 3). Relative (to body weight) testicular weights were similarly affected (data not shown). Additionally, in males there were significant dose-dependent increases (approximately 30%) in adrenal weights, and de-

TABLE 1
BMS-189453: 1-Month Oral Toxicity Study in Rats: Summary of Clinical Pathology Findings (Week 4)

Dose (mg/kg)	Neutrophils (1000/μl)	ALK PHOS (U/l)	ALT (U/l)
Males			
0	1.2 ± 0.2	385.0 ± 25.0	45.0 ± 7.0
15	2.0 ± 0.4[a]	642.0 ± 47.0*,[a]	73.0 ± 9.0[a]
60	3.1 ± 0.6*	738.0 ± 42.0**	101.0 ± 7.0*
240	3.6 ± 0.6*,[a]	1125.0 ± 99.0**,[a]	190.0 ± 105.0**[a]
Females			
0	0.6 ± 0.2[a]	227.0 ± 13.0	34.0 ± 3.0
15	1.2 ± 0.2[a]	566.0 ± 24.0**	59.0 ± 3.0**
60	2.6 ± 1.0	805.0 ± 28.0**	72.0 ± 7.0**
240	2.9 ± 0.6[a]	1094.0 ± 100.0**,[a]	100.0 ± 28.0**,[a]

Note. Mean ± SE, $n = 5$ except where indicated.
[a] $n = 4$
* $p < 0.05$, ** $p < 0.01$.

creases (42 to 48%) in thymus weights at 60 (males) and 240 mg/kg (data not shown). The only clear BMS-189453-related histopathologic changes occurred in the testes and epididymides (Table 2). At all doses, moderate to marked hypospermia occurred in the epididymides, in which ducts were either devoid of sperm or contained necrotic immature and mature spermatids, necrotic cellular debris and globular protein, and edema fluid. Testicular atrophy characterized by moderate to marked degeneration of seminiferous epithelium (tubular atrophy) and moderate interstitial proteinaceous fluid occurred at all doses. Atrophy of seminiferous tubules was a diffuse lesion involving all seminiferous tubules and essentially all germinal cells within each tubule. Sertoli cells were the only viable cells within the seminiferous tubule. Besides Sertoli cells, many seminiferous tubules contained intraluminal globular protein and/or edema fluid. In addition to protein, some tubules contained necrotic spermatids and/or necrotic cellular debris. Although seminiferous tubules were separated from one another by a variable degree of interstitial proteinaceous fluid, Leydig cells were present and appeared unaffected.

One-Week Oral Toxicity Study in Rats

All animals survived to the scheduled necropsies on days 8 and 36. Swelling of the muzzle, limbs, and ears and red discoloration of the skin occurred at 50- and 100-mg/kg groups during the dosing period. Red watery penile discharge was noted at 12.5, 50, and 100 mg/kg. There were no significant differences in mean body weights or food consumption values (data not shown).

At the day-8 necropsy, there were no significant differences in mean testicular weights or epididymal sperm counts between treatment groups (Fig. 4). Minimal decreases (12 to 22%) in mean epididymal sperm counts were evident at 25-mg/kg and higher groups, but did not reach statistical significance. However, following the 1-month drug-free observation period (day-36 necropsy), mean testicular weights and epididymal sperm counts were significantly lower (64 to 66% and 67 to 84%, respectively) than controls at all doses. Relative testicular weights were similarly affected (data not shown).

Histopathologic evaluation of testes at day 8 revealed only minimal degeneration of seminiferous tubules, characterized by necrosis of germinal cells (spermatogonia and spermatocytes), in tubules at all doses (Table 3). Affected germinal cells were shrunken and contained intense cytoplasmic eosinophilia and pyknosis or karyorrhectic nuclei (Fig 5). Additionally, minimal epididymal hypospermia was noted at all doses. In contrast, marked atrophy of the seminiferous tubules was noted at day 36 at all doses. Tubular atrophy consisted of a diffuse lesion involving virtually all seminiferous tubules (Fig. 6). Individual tubules were distinctly diminished in diameter, and, in all but a few tubules of the low-dose rats, germinal cells

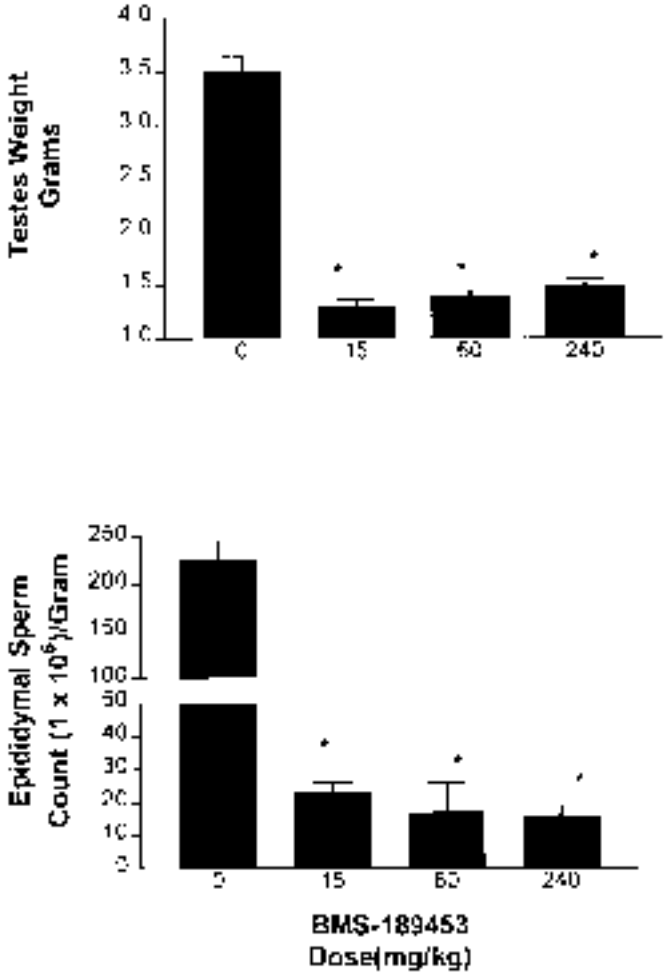

FIG. 3. Effects of 1-month administration of BMS-189453 on testicular weights and epididymal sperm count in rats. Each bar represents the mean ± SE; $n = 10$, except for the 15- and 240-mg/kg groups, where $n = 9$. Asterisks represent significant differences from control as determined by Dunnett's test ($p < 0.05$).

TABLE 2

BMS-189453: 1-Month Oral Toxicity Study in Male Rats: Incidence and Severity of BMS-189453-Related Histopathologic Findings

Dose (mg/kg)	0	15	60	240
Epididymis				
Hypospermia				
Moderate severity	0	4	5	4
Marked severity	0	0	0	1
Testis				
Necrosis				
Moderate severity	0	1	0	0
Marked severity	0	4	5	5
Edema				
Moderate severity	0	4	5	4

Note. Dose (mg/kg) administered to five rats per dosage group.

were nonexistent. At all dose levels, Sertoli cells, which appeared normal, were virtually the only cells remaining within the seminiferous tubules, which were separated from one another, at all dose levels, by proteinaceous interstitial fluid.

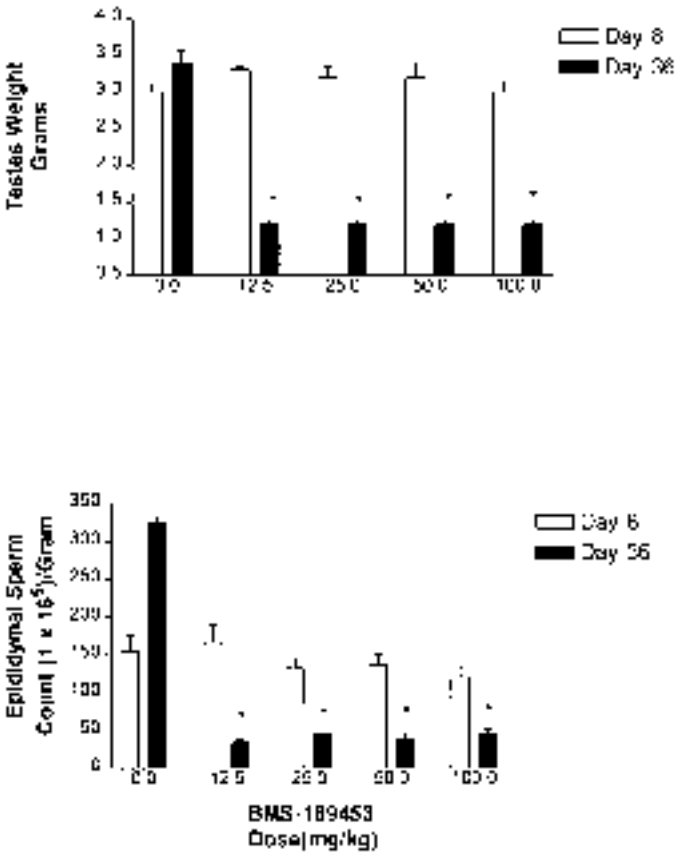

FIG. 4. Effects of 1-week administration of BMS-189453 on testicular weights and epididymal sperm counts in rats necropsied on days 8 or 36. Data presented as in Figure 3 ($n = 5$).

TABLE 3

BMS-189453: 1-Week Oral Toxicity Study in Male Rats: Incidence and Severity of BMS-189453-Related Histopathologic Findings

Dose (mg/kg)	0	12.5	25	50	100
Day 8					
Testis					
Degeneration, spermatogonia					
Minimal severity	0	5	5	5	5
Degeneration, spermatids					
Minimal severity	0	4	4	4	5
Epididymis					
Hypospermia					
Minimal severity	0	4	5	5	5
Day 36					
Testis					
Atrophy, seminiferous epithelium					
Marked severity	0	5	5	5	5
Edema					
Minimal severity	0	3	2	2	1
Moderate severity	0	1	1	3	4
Epididymis					
Hypospermia					
Moderate severity	0	5	5	2	5
Marked severity	0	0	0	3	0

Note. Dose (mg/kg) administered to five rats per dosage group.

Interstitial cells (of Leydig) were present and appeared unaffected. Moderate to marked hypospermia of the epididymides was also noted at all doses.

One-, Three-, and Seven-Day Oral Toxicity Study in Rats

All animals survived to the scheduled necropsies on days 35 and 124. Swelling of the muzzle and limbs occurred within 2 h of dosing at 50 mg/kg on all dosing schedules. There were no significant differences in mean body weights or food consumption values (data not shown).

On the single-dose schedule, significant decreases (57 and 41%, respectively) in mean testicular weights occurred at the day-35 and -124 necropsies at 50 mg/kg only (Fig. 7). On the 3-day dosing schedule, significant decreases (43 and 57%) in mean testicular weights occurred at 10 and 50 mg/kg at the day-35 necropsy, and at 50 mg/kg (63% lower) at the day-124 necropsy. On the 7-day dosing schedule, significant decreases (57 to 64%) in mean testicular weight occurred at all doses at the day-35 and day-124 necropsies. Relative testicular weights were similarly affected (data not shown).

Similar to previous studies, the extent and severity of histopathologic changes in the testes tended to be dependent on dose and duration of treatment. Generally, testes of animals given 2, 10, or 50 mg/kg of BMS-189453 for 1 day were less affected than those dosed for 3 or 7 days (Table 4). Morphologic characteristics of the testicular changes were identical to those described in the previous experiment. Necrosis of the

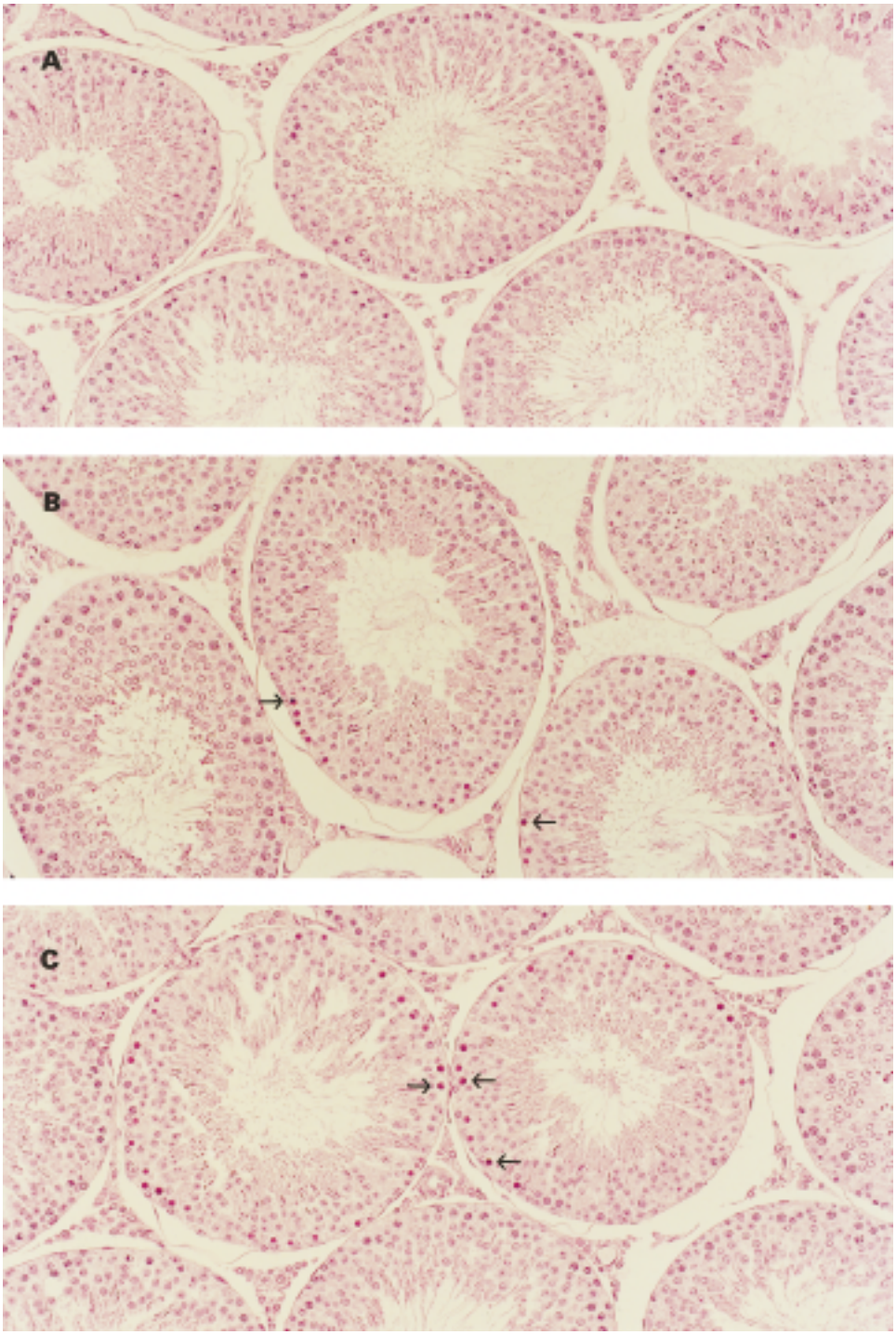

FIG. 5. Photomicrographs (original magnification ×200) of the testicular seminiferous tubules from rats treated for 1 week with BMS-189453 and necropsied on day 8. (A) Control rat; (B) rat treated with 12.5 mg/kg, and (C) rat treated with 100 mg/kg, illustrating minimal effects. Compared to control, there is a slight reduction in the number of germinal cells. The arrows point to necrotic germinal cells (spermatogonium and spermatocytes), which are shrunken and have intensely eosinophilic cytoplasm and pyknotic nuclei.

germinal cells of the testes was the critical drug-induced histopathologic change. With increased time (or duration of treatment) and/or increased dosage, necrosis of germinal cells progressed to degeneration of seminiferous tubules, which in turn progressed to tubular (testicular) atrophy. At the days-35 and -124 necropsies, the most severely affected animals were those that received 50 mg/kg BMS-189453 for 7 days, and the least affected animals were those that received 2 mg/kg for 1 day. The overall incidences of the end-stage lesion, testicular atrophy, were slightly higher at the day-124 necropsy than those observed at the day-35 necropsy, especially at 10 and 50 mg/kg for the single-dose and 3-day-dosing regimens. Mean testicular atrophy severity scores were similar at days 35 and 124 after the 7-day dosing schedule (Fig. 8). However, mean severity

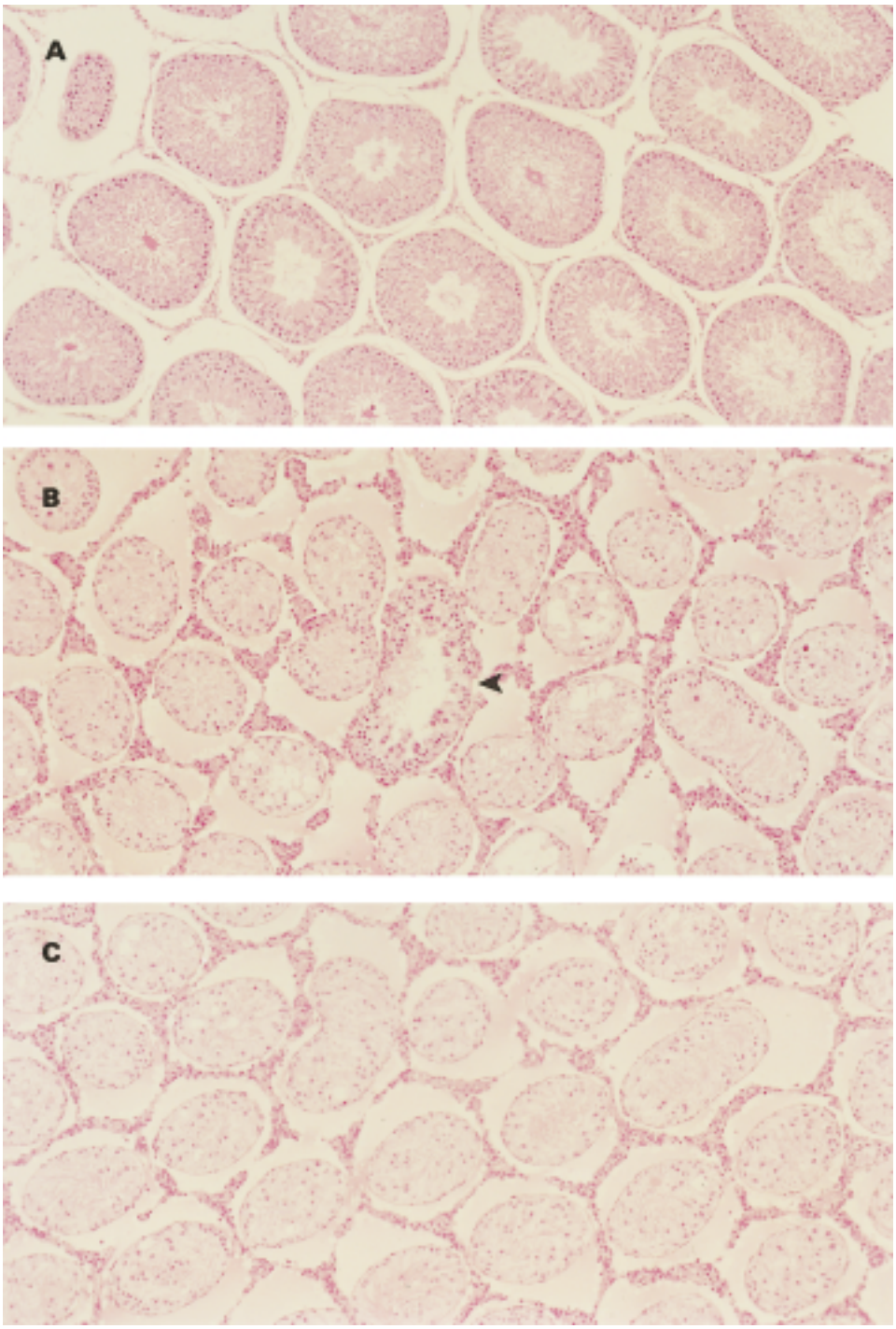

FIG. 6. Photomicrographs (original magnification ×100) of the testicular seminiferous tubules from rats treated for 1 week with BMS-189453 and necropsied on day 36. (A) Control rat, (B) rat treated with 12.5 mg/kg, and (C) rat treated with 100 mg/kg, illustrating marked tubular atrophy. Tubules are reduced in size and the interstitial space contains proteinaceous fluid. At 12.5 mg/kg (B), some tubules (arrowhead) retain germinal cells. At 100 mg/kg (C), virtually all tubules are devoid of germ cells and contain only Sertoli (sustentacular) cells. Compared to control, Leydig (interstitial) cells are relatively crowded because of the reduction in testicular size, but otherwise appear unaffected.

scores were slightly higher at the day-124 necropsy than at the day-35 necropsy for the single-dose regimen at 50 mg/kg and the 3-day dosing regimen at 10 and 50 mg/kg.

One-Week Oral Toxicity Study in Rabbits

All animals survived to the scheduled necropsies on days 8 and 35. There were no BMS-189453-related clinical signs or significant differences in mean body weights or food consumption values (data not shown).

There were no significant differences in mean testicular weights between groups or histopathologic testicular changes at the day-8 necropsy. At the day-35 necropsy, there were no significant differences in mean testicular weights, although one rabbit at 50 mg/kg had a comparatively lower (35%) testis

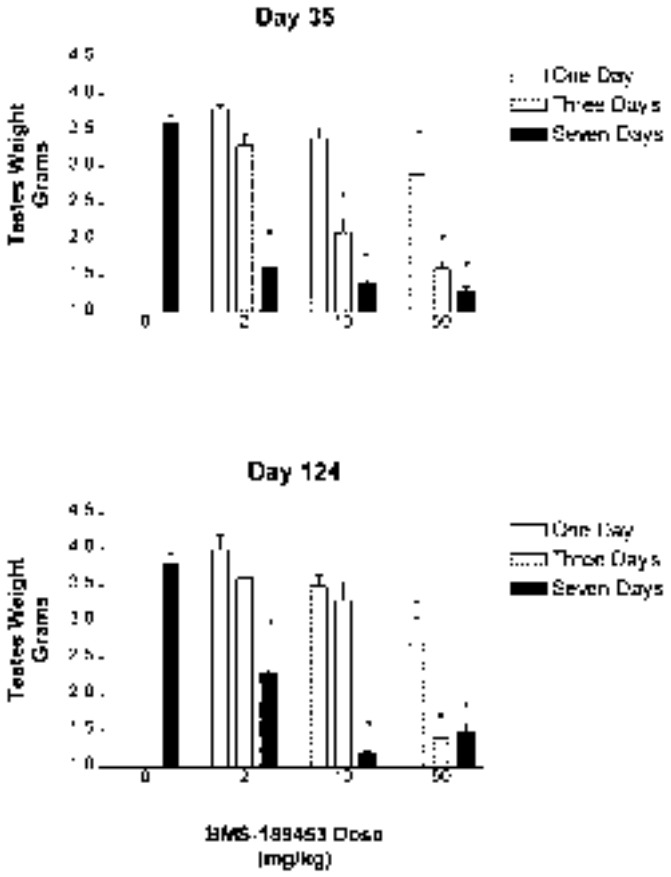

FIG. 7. Effects of 1-, 3-, or 7-day BMS-189453 oral dosing schedules on testicular weights and epididymal sperm count in rats necropsied on days 35 or 124. Data presented as in Figure 3 ($n = 5$).

weight than the control animals (data not shown). At the day-35 necropsy, histopathologic testicular changes included minimal to mild necrosis and degeneration of germinal cells and atrophy of the seminiferous tubules in 2 of 3 rabbits at 50 mg/kg. Affected tubules were generally aspermatogenic. A single layer of Sertoli cells lined the most severely affected tubules. Less affected tubules contained, in addition to Sertoli cells, one or two layers of germinal cells, a variable amount of necrotic cellular debris, and occasional spermatid giant (syncytial) cells (Fig. 9). The lesion was multifocal rather than diffuse. Severity was based upon the number of tubules affected and ranged from 5 to 12% of tubules in the 2 affected rabbits. Epididymides were unaffected.

DISCUSSION

In rats, high doses of the RAR antagonist BMS-189453 produced general signs of toxicity, including poor physical condition, skin erythema, swelling of extremities, and decreased body weight and food consumption. Increases in peripheral-leukocyte counts, serum alkaline phosphatase, and alanine aminotransferase also occurred. In some respects, these general signs of BMS-189453 toxicity are similar to those produced by excessive vitamin A intake or administration of retinoid agonists. For example, erythema is a common clinical sign of toxicity in rodents, following oral administration of vitamin A at 3 to 6 mg/kg, 13-*cis*-retinoic acid at 15 to 20 mg/kg, all trans-retinoic acid at 5 to 50 mg/kg, or etretinate at 10 to 15 mg/kg (Kamm, 1982; Teelmann, 1981). Oral administration of the retinoid agonists SMR2 or SMR6 to mice at doses from 0.1 to 0.4 mg/kg resulted in a 2- to 4-fold elevation in leukocyte counts (Lindamood *et al.*, 1987). Administration of 13-cis- and all trans-retinoic acid to rats or mice at doses ranging from 14 to 300 mg/kg was associated with an increase in serum alkaline phosphatase and testicular degeneration (Hixson *et al*., 1979; Hixson and Denine, 1978; Kurtz *et al*., 1984; Sani and Meeks, 1983).

Similarities between BMS-189453, vitamin A, and retinoid-agonist toxicities were evident; however, specific differences were noted. Although SMR2 and SMR6 induced leukocytosis, they were also associated with leukopoiesis in the spleen and bone marrow, which may have been due to either a direct effect and/or secondary response to subacute inflammatory skin reactions (Lindamood *et al.*, 1987). All trans- and 13-cis-retinoic acid produced increases in alkaline phosphatase that were

TABLE 4

BMS-189453: 1-, 3-, or 7-Day Oral Toxicity Study in Male Rats: Incidence of BMS-189453-Related Testicular Histopathologic Findings

Dose (mg/kg)	0	2	10	50
DAY 35				
Testicular degeneration				
One dose	0	5	5	5
Three doses	0	5	5	5
Seven doses	0	5	5	3
Testicular atrophy				
One dose	0	0	0	2
Three doses	0	0	2	5
Seven doses	0	5	5	5
Testicular edema				
One dose	0	0	0	1
Three doses	0	0	2	5
Seven doses	0	4	5	5
DAY 124				
Testicular degeneration				
One dose	0	0	2	4
Three doses	0	0	2	5
Seven doses	0	4	5	1
Testicular atrophy				
One dose	0	1	2	4
Three doses	0	0	1	5
Seven doses	0	5	5	5
Testicular edema				
One dose	0	0	2	3
Three doses	0	0	1	5
Seven doses	0	4	5	5

Note. Dose (mg/kg) administered to five rats per dosage group.

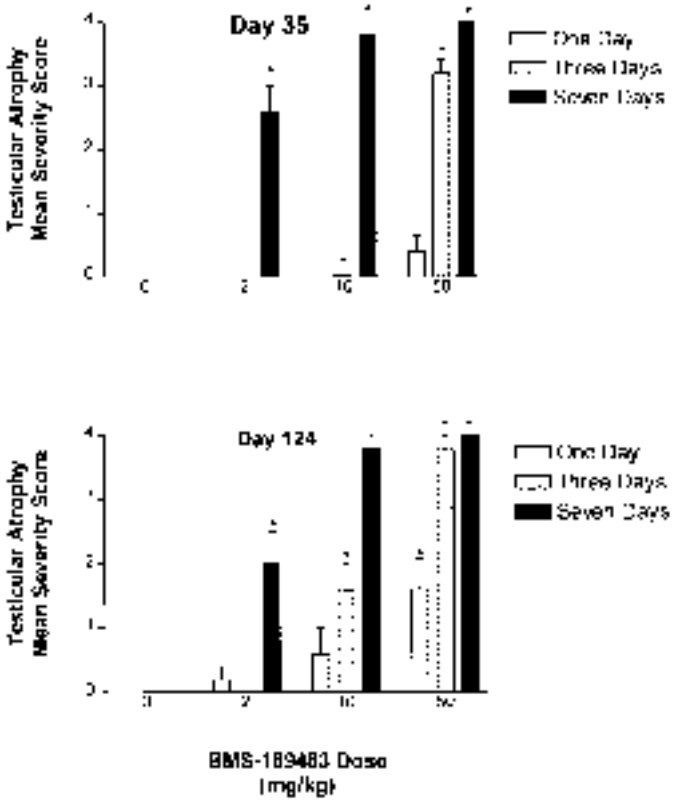

FIG. 8. Effects of 1-, 3-, or 7-day BMS-189453 oral dosing schedules on the severity of testicular atrophy in rats necropsied on days 35 and 124. Asterisks represent significant differences from control as determined by Fisher's exact test ($p \leq 0.05$). Severity scores of minimal, mild, moderate, or marked correspond to severity scores of 1, 2, 3, or 4, respectively.

associated with bone toxicity, whereas administration of retinylidene dimedone (a retinoid agonist) had no effect on alkaline phosphatase, even in the presence of mild bone toxicity (Hixson and Denine, 1978; Kurtz *et al*., 1984). Although increases in leukocyte counts and serum alkaline phosphatase activity occurred with BMS-189453, no microscopic evidence of increased leukopoiesis or bone toxicity was evident. In general, BMS-189453 appeared to affect male rats to a greater extent than females. Sex-related differences in pharmacokinetic or metabolic profiles of BMS-189453 are plausible explanations for this difference; however, these endpoints were not measured in the present experiments. Retinoic acid agonists are also associated with a multiplicity of effects not seen with BMS-189453. For example, retinoid agonists are associated with decreased erythrocyte counts and hyperlipidemia (Hixson *et al*., 1979; Kurtz *et al*, 1984; Sani and Meeks, 1983; Standeven *et al*. 1996a). SMR2 and SMR6 were associated with lesions in the bone, thymus, lymph nodes, spleen, pituitary gland, and stomach. Oral administration of 13-cis- and all-trans-retinoic acids produced inflammation, necrosis, degeneration, and biliary hyperplasia in the livers of rats (Lindamood *et al.*, 1987; 1990; Sani and Meeks, 1983). These effects did not occur with BMS-189453. Although BMS-189453-related elevations in serum ALT levels were noted in rats, no microscopic liver changes were noted.

In rats, the testis was exquisitely sensitive to the effects of BMS-189453. Testicular degeneration/atrophy with epididymal hypospermia occurred at low doses that produced no other signs of toxicity (2 mg/kg for 7 days and 10 mg/kg for 3 days). The testicular effects were progressive with time, relative to dosing, and were long lasting. The early lesion, degeneration of germinal cells, was evident following a 7-day exposure. The initial injury to germinal cells progresses, over time, to testicular atrophy. In rats, the progressive loss of all viable germ cells and the irreversibility of effect are consistent with the hypothesis that the critical insult is to stem cells. In rabbits, BMS-189453 also produced testicular toxicity after 7 daily doses of 50 mg/kg, and although the lesions were somewhat similar, they were less severe than those observed in rats. These findings seem to indicate that this testicular toxicity results from a direct effect of BMS-189453 on the testis and not secondary to a general toxicity.

Vitamin A (retinol/retinoic acid) is essential in maintaining testosterone levels and testicular function and viability (Griswold *et al*., 1989; Mitranond *et al*., 1979; Sobhon *et al*., 1979). The testis and epididymides are known to be rich in RARs, especially within Sertoli cells (Kato *et al*., 1985). Vitamin A deficiency (VAD) results in decreased testicular RAR levels that precipitously increase in Sertoli cells following retinol replenishment. In addition, VAD is associated with testicular apoptosis or selective loss of germ cells in the testes of rats (Akmal *et al*., 1998) and is known to affect the visual system in adult rats (Coward *et al*., 1969). No observable clinical or microscopic changes in the eyes were noted with BMS-189453. However, subtle, drug-related functional changes in the visual system cannot be ruled out.

Similar to BMS-189453-related testicular changes, VAD in male rats causes progressive degeneration or loss of germ cells that is characterized by seminiferous tubules containing mainly Sertoli cells, unlike BMS-189543, however, spermatogonia and a small number of spermatocytes are still present (Huang and Hembree, 1979). In addition, testicular effects of VAD in rats are rapidly reversible with supplementation of retinol or its aldehyde derivative retinyl acetate (Huang and Marshall, 1983). Interestingly, BMS-189453, even after a single dose, appeared to induce a state of "functional VAD in the testes," presumably by antagonizing the effects of retinoic acid at its receptor. This state of functional VAD was expressed as testicular atrophy within 1 month of treatment, and was still present 4 months after treatment was terminated when animals were maintained on a vitamin A-sufficient diet. The BMS-189453-induced functional VAD in the testes does not spare spermatogonia, as is reported with dietary-induced VAD. As expected, BMS-189453-induced testicular toxicity was not specific to the rat since similar, yet less severe, changes were noted in rabbits treated at comparable doses of BMS-189453.

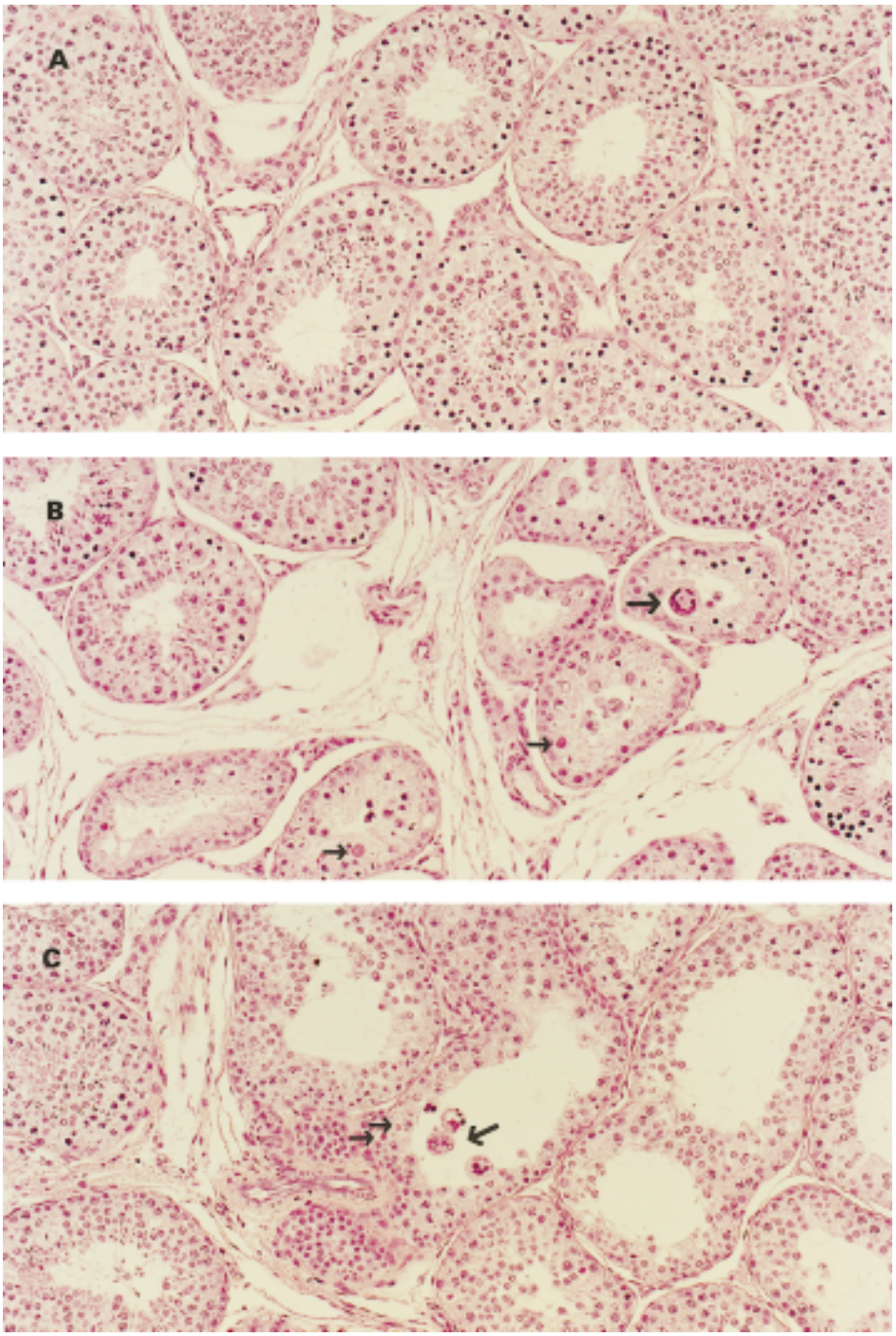

FIG. 9. Photomicrographs (original magnification ×200) of the testicular seminiferous tubules from rabbits treated for 1 week with BMS-189453 and necropsied on days 8 or 36. (A) Control rabbit day 8; (B and C) rabbit treated with 50 mg/kg on day 36, illustrating focal tubular atrophy. Affected tubules have fewer germinal cells, some necrotic germinal cells (thin arrows), and one or more multinucleated spermatids (thick arrow).

Oral administration of retinoid agonists to rats, mice, and dogs produces testicular changes analogous to those seen with BMS-189453; however, repeated administrations are necessary for the expression of toxicity (Kamm, 1982; Kurtz *et al*., 1984; Lindamood *et al*., 1987). As is the case with VAD, retinoid-induced testicular toxicity is reversible following cessation of treatment (Kamm, 1982; Teelmann, 1981). Testicular toxicity and marked decreases in circulating and testicular retinol levels were associated with repeated administration of Ro 23–2895 (a retinoid agonist) to rats (Bosakowski *et al*., 1991). These findings suggest that, in rats, alterations in retinol homeostasis (e.g., functional VAD) may play a significant role in the degenerative testicular changes induced by retinoid agonists.

In conclusion, oral administration of the triple RAR antagonist BMS-189453 to rats produced a spectrum of toxicologically significant effects that, at high doses, share common

properties with those observed after excessive vitamin A intake. At lower doses, BMS-189453 selectively produced progressive testicular toxicity and hypospermia, a result that shares some similar characteristics with vitamin A deficiency (VAD). Unlike VAD, however, BMS-189453-related testicular toxicity resulted in almost complete loss of spermatogonia and was not readily reversible following cessation of treatment. Lastly, the BMS-189453-induced testicular toxicity was not specific to rats, as similar lesions occurred in rabbits under identical experimental conditions of dose and duration.

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