TOXICOLOGICAL SCIENCES **69,** 175–182 (2002)

Microtubules of the Mouse Testis Exhibit Differential Sensitivity to the Microtubule Disruptors Carbendazim and Colchicine

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Received February 15, 2002; accepted April 30, 2002

The testicular toxicant benomyl and its metabolite, carbendazim cause reproductive damage to the rat, an early sign of which is sloughing of germ cells with associated Sertoli cell fragments. However, the sensitivity of other mammalian species to these benzimidazole compounds is not clear. In this study, the effects of carbendazim and colchicine, a known microtubule disruptor, on the mouse seminiferous epithelium were characterized, and the amount of carbendazim reaching the mouse testis was measured. Testes were assessed for histological effects 3 h and 6 h after administration of carbendazim (2000 mg/kg, ip), and 6 h after intratesticular administration of either a low or high dose (5.3 or 117.6 μg/g testis) of colchicine. Carbendazim caused no signs of histological damage to the mouse testis, and the microtubule cytoskeleton was intact and identical to controls based on immunostaining with tyrosinated α tubulin and β tubulin antibodies. Similarly, the seminiferous epithelium of mouse testis was undamaged and the microtubule cytoskeleton was intact after a low dose of colchicine, while a comparable dose of colchicine injected into rat testis caused marked toxicity. However, mouse testes did show microtubule disruption and severe germ cell sloughing after administration of a high dose of colchicine. The amount of carbendazim measured in mouse testis was 375 nmol/g testis, which is higher than the value measured in rat testis after a toxic dose of carbendazim. Therefore, carbendazim reaches the mouse testis at or above levels measured in the rat, yet the mouse is apparently insensitive to this microtubule disrupting agent.

Key Words: **benzimidazoles; carbendazim; colchicine; microtubules and tubulin; microtubule disrupting agents; microtubule associated proteins; Sertoli cells.**

Benomyl is a benzimidazole fungicide commonly used on a variety of food crops and ornamental plants whose fungicidal activity is based on the ability to interfere with the assembly of fungal microtubules (Davidse, 1986). Administration of benomyl or its metabolite, carbendazim, to rats is known to cause reproductive damage, including decreased epididymal and testicular weights, and reduced epididymal sperm counts and fertility (Barnes *et al.*, 1983; Carter and Laskey, 1982; Hess *et al.*, 1991; Linder *et al.*, 1988; Nakai *et al.*, 1992). Histopathology of the testis is characterized by vacuolization of Sertoli cells, and sloughing of elongating spermatids and spermatocytes when damage is severe (Hess and Nakai, 2000). A corresponding decrease in the number of microtubules in Sertoli cells after carbendazim treatment has been observed using electron microscopy and immunohistological techniques (Hess and Nakai, 2000; Nakai and Hess, 1994). While a single dose of benomyl (100 mg/kg) is sufficient to elicit a testicular lesion (Hess *et al.*, 1991), the acute systemic toxicity of benomyl is very low, with an LD_{50} of greater than 10 g/kg in the rat (Sherman *et al.*, 1975).

Carbendazim has been demonstrated to be the active toxicant responsible for the observed testicular toxicity. In a previous study, testicular levels of carbendazim, and not benomyl, were directly correlated with the extent of testicular damage in rats (Lim and Miller, 1997a). Carbendazim was also found to be a two-fold more potent testicular toxicant than was benomyl (Lim and Miller, 1997a). Carbendazim is thought to act as a fungicide by binding to the colchicine binding site of fungal tubulin, resulting in inhibition of microtubule assembly *in vitro* (Davidse, 1986; Davidse and Flach, 1977). It has been proposed that carbendazim causes testicular toxicity in mammals by a similar mechanism that disrupts microtubules (Davidse and Flach, 1977; Russell *et al.*, 1992). Recently, carbendazim was shown to disrupt microtubules in freshly isolated rat seminiferous tubules *in situ*, evidenced by an increase in soluble pools of tubulin (Correa and Miller, 2001). This increase in tubulin subunit levels may link the characterized histological damage (Hess and Nakai, 2000) with the ability of carbendazim to disrupt microtubule assembly *in vitro* (Davidse and Flach, 1977; Friedman and Platzer, 1978; Ireland *et al.*, 1979; Russell *et al.*, 1992; Winder *et al.*, 2001).

Mammalian species exhibit differential sensitivity to the benzimidazoles (Davidse, 1986). In a multigenerational study, chronic treatment of rats and hamsters with carbendazim caused reproductive damage to rats based on several endpoint measurements, yet in hamsters, only sperm measures were

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affected (Gray *et al.*, 1990). In another study that investigated sperm morphology, mouse sperm appeared to be less sensitive to the effects of carbendazim than rat sperm (Evenson *et al.*, 1987). However, early time points and single dose effects of carbendazim were not evaluated in these studies (Evenson *et al.*, 1987; Gray *et al.*, 1990). It is interesting that prepubertal rats have been determined to be less sensitive to the effects of carbendazim compared to adult rats (Carter *et al.*, 1984; Lim and Miller, 1997b). This appears to be due, at least in part, to a relatively low level of detectable carbendazim in the prepubertal testis compared to the adult rat testis (Lim and Miller, 1997b). Clearly, species differences in carbendazim distribution, metabolism, and elimination have to be considered when evaluating species-specific toxicity.

Colchicine, a well described microtubule disruptor, also causes testicular damage in rats, including sloughing, similar to that caused by carbendazim (Allard *et al.*, 1993; Russell *et al.*, 1981). However, the degree to which colchicine causes reproductive damage in the mouse is not well characterized. In one study, injection of colchicine into mouse testes each day for up to 9 days caused sperm abnormalities and disruption of microtubules in the seminiferous epithelium (Handel, 1979).

In the current study, we have investigated the sensitivity of the mouse testis to carbendazim and colchicine, and potential mechanisms underlying any differential effects of these agents compared to the rat. The effects of these two microtubule disruptors on seminiferous tubule structure were characterized and carbendazim levels in the testis were determined. The possibilities that a reduced level of carbendazim reaches the mouse testis or that the mouse cytoskeleton is relatively insensitive to the effects of carbendazim were addressed.

MATERIALS AND METHODS

Animals. Adult male Swiss Webster mice (25–30 g) and adult male Sprague-Dawley rats (350–400 g) were purchased from Charles River Laboratories (Wilmington, MA). The animals were allowed access to water and mouse or rat chow *ad libitum* and were housed in a temperature controlled facility with a 12 h light:12 h dark lighting cycle.

Chemicals. HPLC grade water and methanol were purchased from Fisher Scientific (Pittsburgh, PA). All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise indicated.

Administration of chemicals. For histopathological assessment, carbendazim was injected into mice (ip) at 2000 mg/kg carbendazim suspended in corn oil, and controls were injected with corn oil. The animals were asphyxiated by CO_2 inhalation 2, 3, or 6 h after treatment, and tissues were fixed and processed for microscopy as described below. For determination of testicular levels of carbendazim, 2000 mg/kg carbendazim suspended in corn oil was injected ip and testes were collected 5, 15, 30, 60, 90, and 120 min following injection. For comparison, 164 mg/kg carbendazim suspended in corn oil was injected ip into rats and testes were collected 15 min after injection. Tissues were frozen immediately in liquid nitrogen and stored at −80°C prior to HPLC analysis.

Colchicine was injected intratesticularly into mice at 5.3 or 117.6 μg colchicine/g testis (dissolved in 25 μl PBS), and into rats at 5.6 μg colchicine/g testis (dissolved in 50 μl PBS). The contralateral testis on each animal was injected with a 25 μl (mice) or 50 μl (rat) volume of PBS and served as controls. Animals were sacrificed 6 h after treatment and testes were processed for histology or immunohistochemistry.

Histology. Testes collected after carbendazim and colchicine treatment were immersion fixed in 10% phosphate-buffered formalin and tissue blocks were embedded with glycol methacrylate and sectioned (2.5 μm). Sections were stained by the periodic acid-Schiff reaction and hematoxylin (PAS-H) and viewed by routine light microscopy. Histopathological damage was assessed by examination of 100 seminiferous tubule cross sections per testis. Sections were evaluated for the following endpoints: vacuolization (appearance of vacuoles in the seminiferous epithelium), and sloughing (loss of the apical portions of Sertoli cells and attached germ cells). The percentage of normal tubules and of tubules with the described histological endpoints was determined.

Immunohistochemistry. Testes collected after carbendazim and colchicine treatment were immersion fixed in 1 or 4% paraformaldehyde and tissue blocks were processed for paraffin sectioning. Sections were incubated in 0.3% hydrogen peroxide in methanol for 15 min to block endogenous peroxidase activity, followed by a 10-min incubation in 10% normal goat serum to prevent nonspecific binding of antibodies. Sections were incubated with either anti-tyrosinated α tubulin monoclonal antibody (diluted 1:1000, TUB-1A2, Sigma) or anti-β tubulin monoclonal antibody (diluted 1:100, Chemicon International, Temecula, CA) for 2 h at room temperature or overnight at 4°C. Control sections were incubated as above without primary antibody. All sections were incubated with biotinylated goat anti-mouse IgG secondary antibody (diluted 1:100, DAKO, Carpinteria, CA) followed by incubation with avidin-biotinylated peroxidase complex (Vectastain ABC kit, Vector laboratories, Burlingame, CA). Positive reactions were visualized with diaminobenzidine and hydrogen peroxide.

Carbendazim levels in the testis. Mice testes were collected at 5, 15, 30, 60, 90, and 120 min postinjection, and rat testes were collected 15 min postinjection, as described above. Testes from each animal were weighed and homogenized in two volumes of 50 mM potassium phosphate buffer, pH 7.4, using a glass homogenizer and a teflon pestle. Homogenates were mixed with two volumes of methanol, vortexed for 1 min, and centrifuged at 2000 × *g* for 10 min at 4°C. The supernatants were removed and 100 μl aliquots were analyzed by HPLC based on the method of Lim and Miller (1997b). Carbendazim was separated by reverse-phase HPLC on a C18 column using a gradient solvent system. The flow rate was 1.0 ml/min, and detection was by UV absorption at 280 nm. Initial conditions in the gradient solvent system were 70% water: 30% methanol, followed by a linear gradient to 30% water:70% methanol over 5 min. This ratio of solvents was maintained for 5 min, followed by a linear gradient back to 70%:30% over 5 min and subsequent equilibration for an additional 15 min. The retention time for carbendazim was 13.7 min.

Standards ranging in concentration from 50 to 1600 ng carbendazim in 100 μl were made up in the same buffer:methanol ratio as testis samples, and used to construct standard curves of concentration versus area under the curve. The nmoles of carbendazim in testis samples were calculated from standard curves.

Statistics. Statistical significance of treatment effects was determined with a Student's two tailed *t*-test; p values < 0.05 were considered to be statistically significant.

RESULTS

Histology of the Mouse Testis after Treatment with Carbendazim and Colchicine

Carbendazim was administered to mice in order to determine if this microtubule disruptor affects the mouse testis. A testis section from a control mouse obtained 3 h after injection of PBS (Fig. 1A) contained an array of developing germ cells with spermatids adjacent to the lumen of the seminiferous tubules and an intact seminiferous epithelium. Testis sections

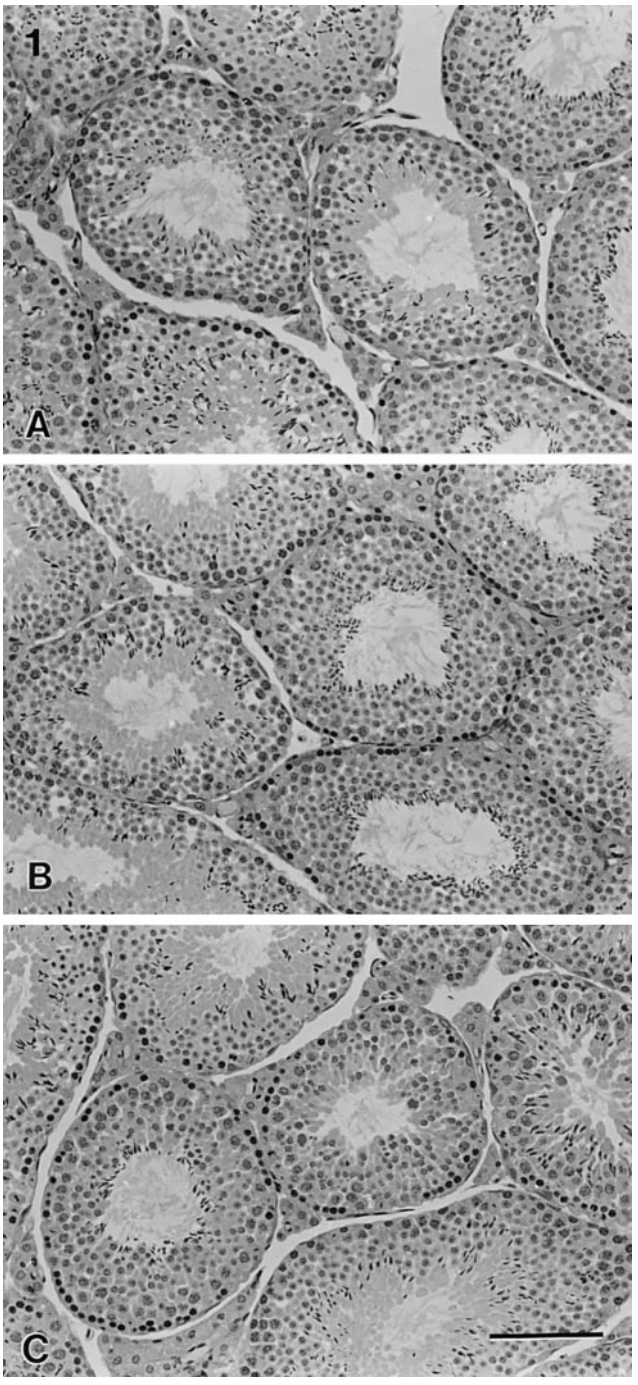

FIG. 1. Histological effects of carbendazim on the mouse testis. Mice were injected with 2000 mg/kg carbendazim, and testes were collected and processed for PAS-H staining (Bars = 100 μm). (A) A control testis obtained 3 h after ip injection of PBS shows the normal seminiferous epithelial structure. Testes obtained 3 h (B) and 6 h (C) after injection of carbendazim appear normal and identical to the control.

from mice treated with carbendazim (2000 mg/kg), at both 3 h (Fig. 1B) and 6 h (Fig. 1C) timepoints, were indistinguishable from the control, with no evidence of damage or sloughing of germ cells. The seminiferous epithelium showed no sign of vacuolization and was comparable to control sections. The dose of carbendazim used (2000 mg/kg) was approximately 10 times that which caused massive sloughing in the rat testis (~200 mg/kg; Hess *et al.*, 1991; Nakai *et al.*, 1992). Similarly, mice injected with 1000 mg/kg, or a level equivalent to that which induces sloughing in rats (164 mg/kg; Lim and Miller, 1997a), showed no evidence of testicular damage in this study (data not shown). As a positive control, rat testes prepared after injection of carbendazim (164 mg/kg) displayed massive sloughing, while the seminiferous epithelium of control rats remained intact (data not shown). Thus, the mouse seminiferous epithelium appears to be insensitive to the effects of carbendazim even at very high dose levels.

To determine if the insensitivity of the mouse testis to carbendazim reflects an insensitivity to microtubule disrupting agents in general, colchicine, a well-known microtubule disruptor, was injected intratesticularly. A representative section of a control testis that was injected with PBS is shown in Figure 2A; the seminiferous epithelium is intact and is similar to the control in Figure 1A. Figure 2B contains a testis section from a mouse injected with colchicine at a dose (5.3 μg/g testis) that caused massive sloughing in rat testis in the present study (data summarized in Table 1). No vacuolization or sloughing was detectable above control levels at this dose and the epithelia were comparable to controls. However, the tubule lumens appeared to be larger than in control sections and some of the spaces between groups of cells were larger than in controls. In contrast, mice testes that were injected with 117.6 μg colchicine/g testis (approximately 20 times the dose that caused extensive damage in rats) showed massive damage in the majority of the seminiferous tubules. In the testis section shown (Fig. 2C), germ cells with attached apical epithelia are completely detached from the basal epithelium. The sloughed germ cells in the lumen of tubules consist of spermatocytes or spermatids depending on the stage shown in a specific cross-section. This reflects one of the characteristics of sloughing, i.e., that detachment occurs between dissimilar cohorts of germ cells. Based on these results, the mouse testis appears to be virtually insensitive to the disrupting effects of colchicine at a dose which damages rat testis, yet exhibits extensive damage after higher dose levels of colchicine.

The histological effects of colchicine treatment are summarized in Table 1. Testis sections from mice injected with a low dose of colchicine were essentially normal, while those from mice given a high dose had a significant decrease in the number of normal tubules (38.0% $\pm$ 12.9) compared to control mice (98.0% $\pm$ 0.8). The decrease in normal tubules observed was due to a concommitant increase in the incidence of sloughing. Rats were injected with colchicine (5.6 μg/g testis) as positive controls. Tissue sections examined from these animals exhibited massive sloughing corresponding with a significant de-

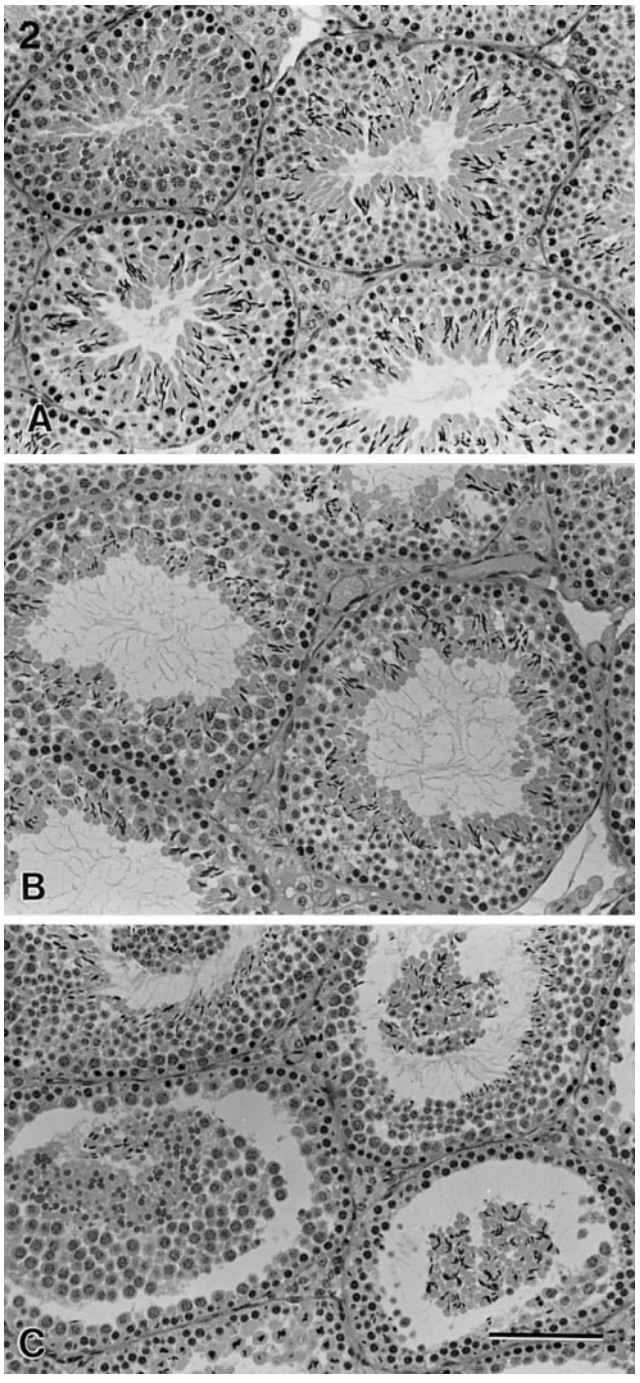

FIG. 2. Histological effects of colchicine on the mouse testis. Mice were injected intratesticularly with colchicine, and testes were collected 6 h postinjection and processed for PAS-H staining (Bars = 100 μm). (A) A control testis that was injected with PBS shows the normal structure. (B) and (C) contain testis sections that were injected with 5.3 μg/g testis or 117.6 μg/g testis, respectively. The lumen is enlarged (B), while massive sloughing of germ cells is seen in the testis treated with a high dose of colchicine (C).

crease in the number of normal seminiferous tubules compared to controls injected with PBS (98.1% ± 1.0 in controls vs. 8.7% ± 4.1 for treated; Table 1).

Effects of Carbendazim and Colchicine on Sertoli Cell Microtubules

To determine if mouse Sertoli cell microtubules are sensitive to carbendazim, mouse testis sections were stained with either tyrosinated α tubulin or β tubulin antibodies. In Figure 3, testis sections obtained 2 h after injection of carbendazim (2000 mg/kg) or control are shown. In the control (Fig. 3A), α tubulin occurs in tubule cross-sections in a spoke-like pattern characteristic of the prominent microtubules of the Sertoli cell cytoskeleton. Virtually identical staining patterns of tyrosinated α tubulin are apparent in seminiferous tubule sections from a mouse testis that was treated with carbendazim (Fig. 3B). The "spokes" observed reflect microtubules extending from the basal to the apical portion of Sertoli cells, perpendicular to the basement membrane of each seminiferous tubule. Similarly, the characteristic pattern of α tubulin staining was observed in tubule sections prepared from mice 6 h after injection of carbendazim (data not shown). Figure 4 contains testis sections obtained 2 h after injection of carbendazim that were stained for β tubulin. Control (Fig. 4A) and treated (Fig. 4B) testes are virtually identical and reveal the pattern of tubulin staining characteristic of the extensive microtubule network of Sertoli cells. Sections prepared from testes 6 h after injection of carbendazim also displayed β tubulin staining indistinguishable from controls (data not shown). The slight differences in staining between adjacent seminiferous tubule sections in Figures 3 and 4 reflect the various stages that were bisected in the testis sections pictured. The immunohistochemical results are in agreement with the histological data, thus supporting the hypothesis that carbendazim does not induce sloughing or loss of microtubule structure in the mouse testis.

The sensitivity of the mouse Sertoli cell cytoskeleton to colchicine was also investigated. Testis sections obtained from mice 6 h after injection of PBS or colchicine (117.6 μg/g testis) were stained for tyrosinated α tubulin and are shown in Figure

TABLE 1
Histological Effects of Colchicine on Rat and Mouse Testis

Animal/treatment	Normal tubules	Vacuolization	Sloughing
Rat			
PBS control	98.1 ± 1.0	1.9 ± 1.0	0
5.6 μg col/g ts	8.7 ± 4.1*	5.8 ± 3.0	85.5 ± 8.1*
Mouse			
PBS control	98.0 ± 0.8	0.8 ± 0.2	1.2 ± 0.7
5.3 μg col/g ts	99.0 ± 0.6	0.0 ± 0.0	1.0 ± 0.6
117.6 μg col/g ts	38.0 ± 12.9*	0.3 ± 0.2	62.0 ± 13.0*

Note. Mice and rats were injected intratesticularly with the indicated dose of colchicine and testes were processed 6 h postinjection. Histological endpoints were assessed by examination of 100 seminiferous tubule cross sections per testis. Col, colchicine; ts, testis. Values given are percentages. Each value represents the mean ± SEM; $n = 3$ for rat control and treatment; $n = 13$, 4, and 10 for mice control, low dose, and high dose, respectively.

*Significantly different from control group ($p < 0.001$).

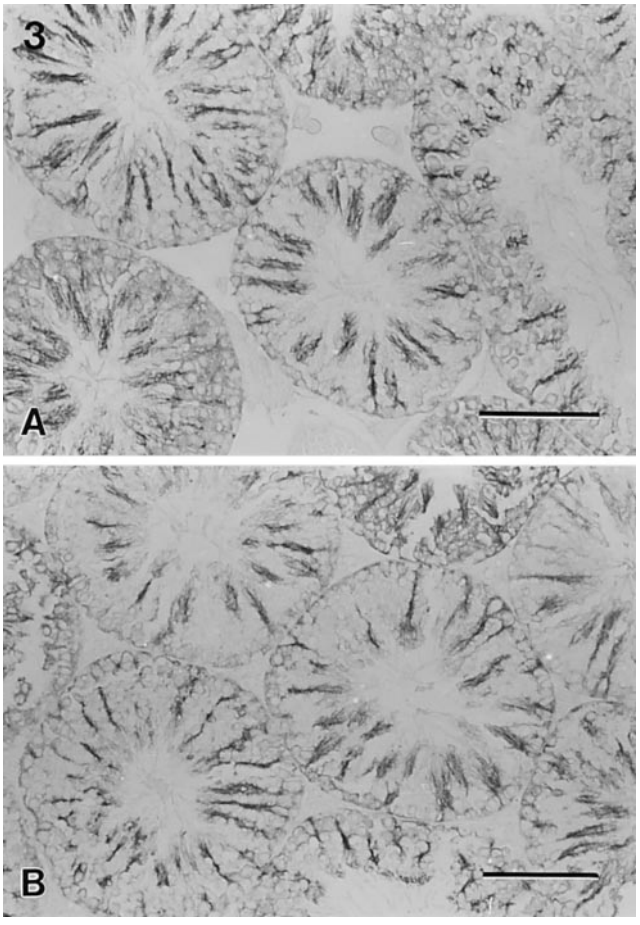

FIG. 3. Effect of carbendazim on α tubulin in the mouse seminiferous epithelium. Testes were collected 2 h after ip injection of 2000 mg/kg carbendazim, processed, and stained for tyrosinated α tubulin. Bars = 100 μm. In (A), a control testis section from a mouse that was injected with PBS is shown, while (B) contains a testis section from a treated mouse. Both sections show the typical, spoke-like staining pattern of α tubulin in Sertoli cells.

5. In the control (Fig. 5A), α tubulin staining appears similar to that seen in Figure 3A, with spokes of dark staining representing the microtubules of the intact Sertoli cell cytoskeleton. Testis sections from mice that were injected with a low dose of colchicine (5.3 μg/g testis) exhibited a similar pattern of α tubulin staining as the control, with characteristic bands of immunoreactivity (data not shown). However, most tubules in testis sections from mice injected with a high dose of colchicine (117.6 μg/g testis) exhibited a dramatic disruption of the microtubule network of Sertoli cells (Fig. 5B). Sloughed material in tubule lumens was relatively diffusely stained, with the exception of very small areas of "spoke-like" staining. Results were similar in testis sections from colchicine treated mice that were immunostained with the β tubulin antibody, i.e., tubule sections contained diffusely stained, sloughed material, and degenerating cells (data not shown). Thus the sloughing of germ cells after injection of a high dose of colchicine in mice occurs concurrently with the disruption of the prominent microtubule network of Sertoli cells, similar to results from rat studies (Allard *et al.*, 1993; Russell *et al.*, 1981).

Testicular Levels of Carbendazim in the Mouse

In order to investigate potential mechanisms for the differential sensitivity of the mouse to microtubule disrupting agents, the amount of carbendazim in the mouse testis was measured. Specifically, the goal was to determine if the lack of sensitivity of the mouse seminiferous epithelium to carbendazim could be a result of low levels of this chemical reaching the testis. Carbendazim was injected (2000 mg/kg) into mice, and testes were collected at several time points and processed for HPLC. Figure 6 shows the average nmoles of carbendazim per g testis weight versus time of exposure. A time course of measured carbendazim levels in the rat testis is included in

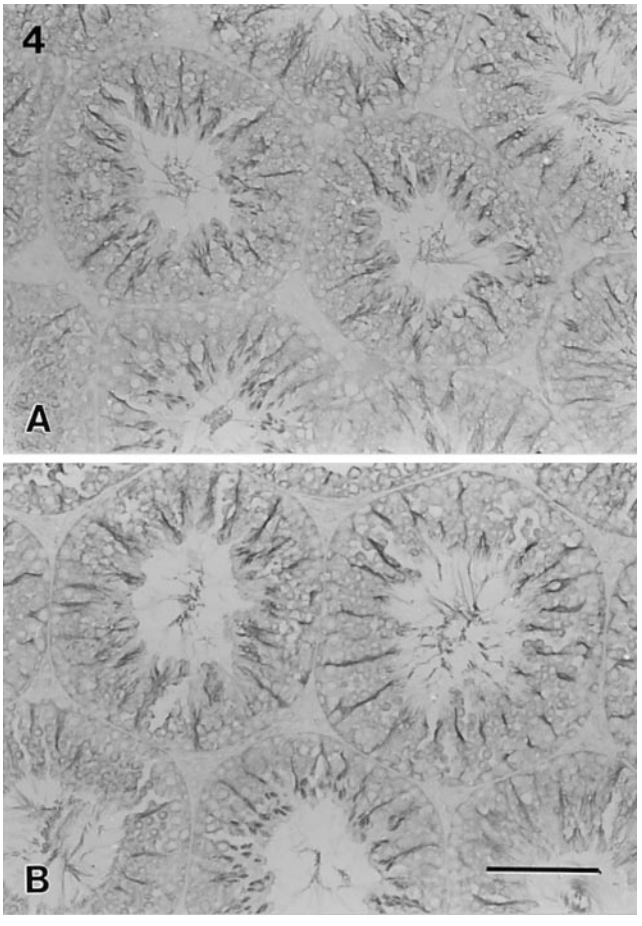

FIG. 4. Effect of carbendazim on β tubulin in the mouse seminiferous epithelium. Testes were collected 2 h after ip injection of 2000 mg/kg carbendazim, processed and stained for β tubulin. Bars = 100 μm. In (A), a control testis section from a mouse that was injected with PBS is shown; (B) contains a testis section from a treated mouse. Note that both sections exhibit the same β tubulin staining pattern.

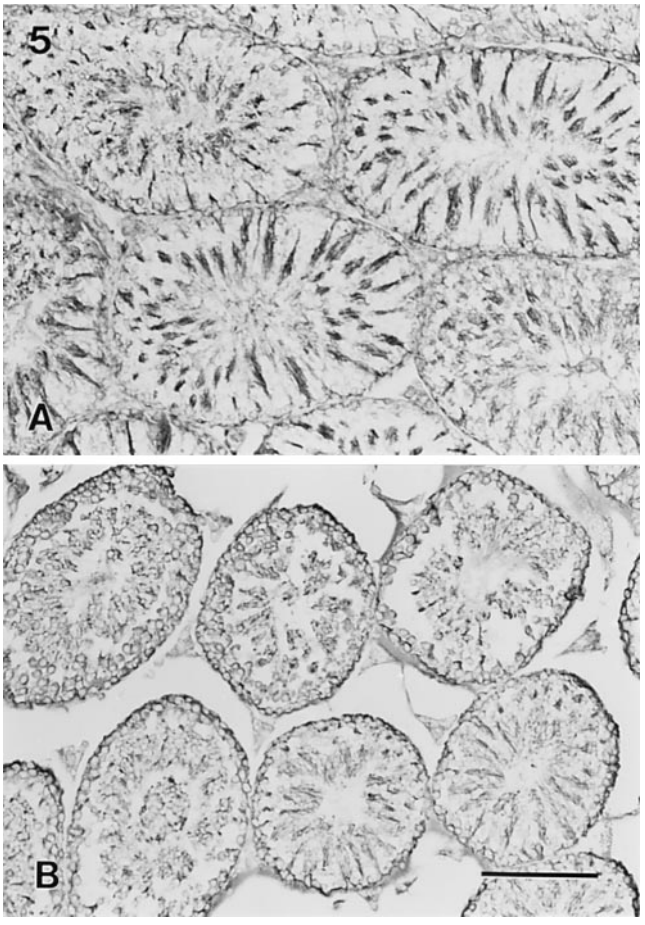

FIG. 5. Effect of colchicine on α tubulin in the mouse seminiferous epithelium. Testes were collected 6 h after intratesticular injection of colchicine and processed for immunohistochemistry using an antibody specific for tyrosinated α tubulin. Bars = 100 μm. In (A), a control testis section from a mouse that was injected with PBS is shown, while (B) contains a section from a mouse testis obtained after injection of 117.6 μg/g testis. The spoke-like staining pattern exhibited in the control testis (A) has disappeared in the colchicine-treated testis (B).

Figure 6 for comparison (data from Lim and Miller, 1997a). The maximum level of carbendazim detected was 375 ± 117 nmol/g testis, followed by a decline over time. The time of maximum exposure in mice occurred 5 min postinjection, while the maximum level of exposure in the rat was previously determined to occur 15 min postinjection (Fig. 6). Although carbendazim levels declined rapidly over the time period measured, the level detected at 2 h was still relatively high (~100 nmol/g testis) in mouse testis compared to rat testis (~50 nmol/g testis). Nonetheless, the profile of measured carbendazim levels over time in the rat and mouse testis are strikingly similar. Based on our results, carbendazim reaches the mouse testis at or above levels previously detected for the rat, yet does not cause damage to seminiferous tubule structure or disruption of the Sertoli cell microtubule network.

DISCUSSION

The histological damage that occurs in the rat testis after administration of carbendazim (Barnes *et al.,* 1983; Carter and Laskey, 1982; Hess *et al.,* 1991; Linder *et al.,* 1988; Nakai *et al.,* 1992) or colchicine (Allard *et al.,* 1993; Russell *et al.,* 1981) has been well described and is characterized by sloughing of germ cells and attached apical seminiferous epithelium. However, the sensitivity of other mammals to these microtubule-disrupting agents is more variable and not as well understood. In this study, species differences in the degree of sensitivity of the mouse seminiferous epithelium to carbendazim and colchicine were investigated to begin to understand the mechanism underlying the testicular toxicity of these agents. We found that carbendazim caused no apparent histological damage to the mouse seminiferous epithelium even when administered at 10 times the amount that causes massive sloughing in the rat testis. In contrast, colchicine induced sloughing and disrupted the microtubule network of Sertoli cells in the mouse seminiferous epithelium, albeit at a much higher concentration than previously used in the rat (Allard *et al.,* 1993; Russell *et al.,* 1981). Our results demonstrate that the mouse seminiferous epithelium is insensitive to the testicular toxicant,

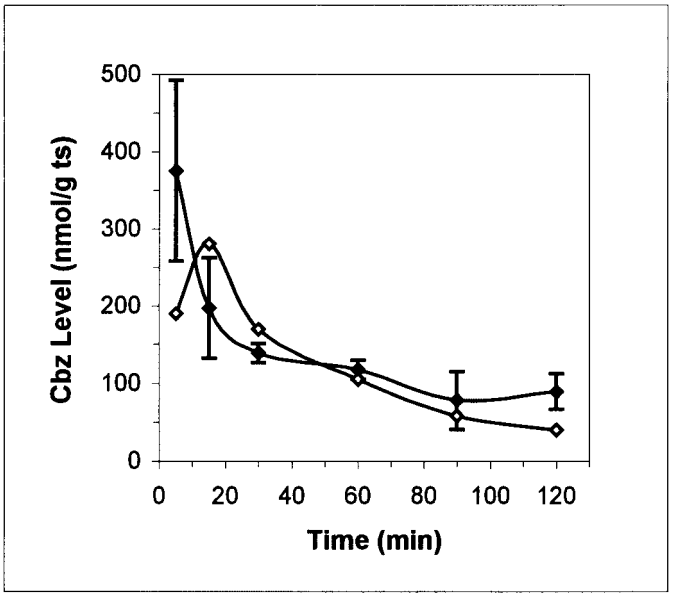

FIG. 6. Time course of carbendazim levels in the mouse testis. Mice were injected ip with 2000 mg/kg carbendazim in corn oil, and testes were collected 5, 15, 30, 60, 90, and 120 min after injection. Testes were homogenized, centrifuged, and supernatants were separated by reverse-phase HPLC (as described in the Methods section). Carbendazim was detected by UV absorption at 280 nm, and the nmoles of carbendazim in testis samples were determined. Mice values (closed diamond) are expressed as the mean carbendazim concentration (nmol/g testis) ± SEM; $n = 4$. Rat values are shown for comparison (open diamond; as previously reported by Lim and Miller, 1997a) and are given as the mean carbendazim concentration ($n = 3$). Cbz, carbendazim.

carbendazim, and is much less sensitive to the disrupting effects of colchicine than previously described for the rat.

In the rat, a single injection of 164 mg/kg carbendazim is capable of eliciting testicular toxicity, with evidence of detachment and sloughing of germ cells beginning as early as 1 h after treatment (Lim and Miller, 1997a) and continuing for several hours. In the present study, the seminiferous epithelium was undamaged and identical to controls at 3 h and 6 h after injection of carbendazim to mice. The extensive microtubule network characteristic of Sertoli cells was also intact based on immunohistochemical staining of tyrosinated α and β tubulin, in agreement with the histological results. In contrast, it has been reported that the rat Sertoli cell microtubule cytoskeleton is severely disrupted after carbendazim treatment, evidenced by loss of tyrosinated α tubulin staining (Hess and Nakai, 2000). The reason why the rat testis is sensitive to carbendazim while the mouse is unaffected even at very high doses is unknown.

There are at least two possible hypotheses to explain why the mouse seminiferous epithelium is insensitive to the microtubule disruptor carbendazim: either carbendazim does not enter the testis (or does so at very reduced amounts), or it does enter the testis and the mouse possesses a mechanism to prevent toxicity. To investigate these possibilities, the level of carbendazim in whole mouse testis homogenates was determined over a 2-h period using HPLC. The maximal concentration of carbendazim measured in the testis, 375 nmol/g testis, occurred at approximately 5 min postinjection with the level decreasing over time as carbendazim was metabolized. This value is comparable to a previously determined maximum value for carbendazim (280 nmol/g testis) in the rat testis, measured 15 min postinjection (Lim and Miller, 1997a). The result of this difference is a relatively longer exposure time in the rat testis to high levels of carbendazim, which might conceivably allow more testicular damage to occur. However, the detectable level of carbendazim in mouse testis at 2 h of exposure was approximately twice as high as previously determined for the rat (Lim and Miller, 1997a). Whether these differences in carbendazim absorption and metabolism contribute to differential sensitivities to this microtubule-disrupting agent will require further investigation. Nevertheless, it is apparent that carbendazim is present in the mouse testis at concentrations greater than or equal to that which was present in the rat testis, yet it does not cause testicular damage.

In contrast to the lack of effect of carbendazim, colchicine did cause damage to the mouse seminiferous epithelium, including germ cell sloughing and loss of Sertoli cell microtubules based on the loss of thick bundles or "spokes" of tyrosinated α tubulin and β tubulin. However, the amount of colchicine necessary to elicit testicular damage was approximately 20 times higher than previously described for the rat. When a dose of colchicine that causes extensive damage in rat testis was administered to mice, testis sections appeared normal except that seminiferous tubule lumens were larger than in controls (Fig. 2B). Since colchicine was injected directly into mice testes, the question of whether it is present in the testis or not is ruled out. Thus, there must be another mechanism at play that contributes to the reduced sensitivity of the mouse testis toward colchicine. For example, the high sensitivity of the rat to the effect of colchicine could be the result of greater binding affinity at the colchicine binding site of tubulin. Moreover, the same could be true for carbendazim since it also interacts at the colchicine binding site. Altogether, the data demonstrate that the mouse seminiferous epithelium is less sensitive than the rat to microtubule disruption by either colchicine or carbendazim.

Overall, our present study has demonstrated that the testicular toxicity of two microtubule disruptors, carbendazim and colchicine, can be very different between species. Moreover, it was previously shown that the effects of carbendazim in the rat are specific for the testes (Sherman *et al.,* 1975) and, within the testis, damage is found only during specific stages of spermatogenesis (Hess and Nakai, 2000). Early studies demonstrated that some benzimidazoles inhibit assembly of brain microtubules *in vitro,* although with much less sensitivity compared to colchicine and other known microtubule-disrupting agents (Friedman and Platzer, 1978; Ireland *et al.,* 1979). It is possible that the mechanisms operating to protect the brain and particular stages of spermatogenesis could be protecting the mouse testis from carbendazim-induced disruption of microtubules. Previous work from this laboratory has shown that addition of microtubule associated proteins (MAPs) to *in vitro* preparations of rat MAP-free tubulin abolished the inhibitory effect of carbendazim on microtubule assembly (Winder *et al.,* 2001). Therefore, it is reasonable to propose that the sensitive stages of spermatogenesis in the rat could have a deficiency of protective MAPs, i.e., one or more MAPs that block the ability of carbendazim to bind tubulin. Perhaps the brain and mouse testis maintain a protective profile of MAPs and thus are insensitive to microtubule disruption after carbendazim treatment. These and other possibilities need further investigation in order to more clearly define the mechanisms underlying the species-specific and tissue-specific sensitivity toward the microtubule disrupting agents, carbendazim and colchicine.

ACKNOWLEDGMENTS

This work was supported by NIH grants RO1 ESO 7832 and PO1 ESO 5707 (Center for Environmental Health Sciences).

REFERENCES

Allard, E. K., Johnson, K. J., and Boekelheide, K. (1993). Colchicine disrupts the cytoskeleton of rat testis seminiferous epithelium in a stage-dependent manner. *Biol. Reprod.* **48,** 143–153.

Barnes, T. B., Verlangieri, A. J., and Wilson, M. C. (1983). Reproductive toxicity of methyl-1-(butylcarbamoyl)-2-benzimidazole carbamate (benomyl) in male Wistar rats. *Toxicology* **28,** 103–115.

Carter, S. D., Hein, J. F., Rehnberg, G. L., and Laskey, J. W. (1984). Effect of

benomyl on the reproductive development of male rats. *J. Toxicol. Environ. Health* **13,** 53–68.

Carter, S. D., and Laskey, J. W. (1982). Effect of benomyl on reproduction in the male rat. *Toxicol. Lett.* **11,** 87–94.

Correa, L. M., and Miller, M. G. (2001). Microtubule depolymerization in rat seminiferous epithelium is associated with diminished tyrosination of α-tubulin. *Biol. Reprod.* **64,** 1644–1652.

Davidse, L. C. (1986). Benzimidazole fungicides: Mechanism of action and biological impact. *Ann. Rev. Phytopathol.* **24,** 43–65.

Davidse, L. C., and Flach, W. (1977). Differential binding of methyl benzimidazol-2-yl carbamate to fungal tubulin as a mechanism of resistance to this antimitotic agent in mutant strains of *Aspergillus nidulans*. *J. Cell Biol.* **72,** 174–193.

Evenson, D. P., Janca, F. C., and Jost, L. K. (1987). Effects of the fungicide methyl-benzimidazol-2-yl carbamate (MBC) on mouse germ cells as determined by flow cytometry. *J. Toxicol. Environ. Health* **20,** 387–399.

Friedman, P. A., and Platzer, E. G. (1978). Interaction of anthelmintic benzimidazoles and benzimidazole derivatives with bovine brain tubulin. *Biochim. Bioph. Acta* **544,** 605–614.

Gray, L. E., Jr., Ostby, J., Linder, R., Goldman, J., Rehnberg, G., and Cooper, R. (1990). Carbendazim-induced alterations of reproductive development and function in the rat and hamster. *Fundam. Appl. Toxicol.* **15,** 281–297.

Handel, M. A. (1979). Effects of colchicine on spermiogenesis in the mouse. *J. Embryol. Exp. Morphol.* **51,** 73–83.

Hess, R. A., Moore, B. J., Forrer, J., Linder, R. E., and Abuel-Atta, A. A. (1991). The fungicide benomyl (methyl 1-(butylcarbomyl)-2-benzimidazolecarbamate) causes testicular dysfunction by inducing the sloughing of germ cells and occlusion of efferent ductules. *Fundam. Appl. Toxicol.* **17,** 733–745.

Hess, R. A., and Nakai, M. (2000). Histopathology of the male reproductive system induced by the fungicide benomyl. *Histol. Histopathol.* **15,** 207–224.

Ireland, C. M., Gull, K., Gutteridge, W. E., and Pogson, C. I. (1979). The interaction of benzimidazole carbamates with mammalian microtubule protein. *Biochem. Pharmacol.* **28,** 2680–2682.

Lim, J., and Miller, M. G. (1997a). The role of the benomyl metabolite carbendazim in benomyl-induced testicular toxicity. *Toxicol. Appl. Pharmacol.* **142,** 401–410.

Lim, J., and Miller, M. G. (1997b). Role of testis exposure levels in the insensitivity of prepubertal rats to carbendazim-induced testicular toxicity. *Fundam. Appl. Toxicol.* **37,** 158–167.

Linder, R. E., Rehnberg, G. L., Strader, L. F., and Diggs, J. P. (1988). Evaluation of reproductive parameters in adult male Wistar rats after subchronic exposure (gavage) to benomyl. *J. Toxicol. Environ. Health* **25,** 285–298.

Nakai, M., and Hess, R. A. (1994). Morphological changes in the rat Sertoli cell induced by the microtubule poison carbendazim. *Tissue Cell* **26,** 917–927.

Nakai, M., Hess, R. A., Moore, B. J., Guttroff, R. F., Strader, L. F., and Linder, R. E. (1992). Acute and long-term effects of a single dose of the fungicide carbendazim (methyl 2-benzimidazole carbamate) on the male reproductive system in the rat. *J. Androl.* **13,** 507–518.

Russell, G. J., Gill, J. H., and Lacey, E. (1992). Binding of [^{3}H]benzimidazole carbamates to mammalian brain tubulin and the mechanism of selective toxicity of the benzimidazole anthelmintics. *Biochem. Pharmacol.* **43,** 1095–1100.

Russell, L. D., Malone, J. P., and MacCurdy, D. S. (1981). Effect of the microtubule disrupting agents, colchicine and vinblastine, on seminiferous tubule structure in the rat. *Tissue Cell* **13,** 349–367.

Sherman, H., Culik, R., and Jackson, R. A. (1975). Reproduction, teratogenic, and mutagenic studies with benomyl. *Toxicol. Appl. Pharmacol.* **32,** 305–315.

Winder, B. S., Strandgaard, C. S., and Miller, M. G. (2001). The role of GTP binding and microtubule-associated proteins in the inhibition of microtubule assembly by carbendazim. *Toxicol. Sci.* **59,** 138–146.