Reproductive Toxicology, Vol. 4, pp. 215–222, 1990
Printed in the U.S.A.

0890-6238/90 $3.00 + .00

ANGUIDINE-INDUCED TESTICULAR INJURY IN LEWIS RATS

MICHAEL W. CONNER, BARBARA H. CONNER, ADRIANNE E. ROGERS, and PAUL M. NEWBERNE

Department of Pathology, Boston University School of Medicine, Boston, Massachusettes

Abstract — Anguidine (diacetoxyscirpenol, DAS) and other trichothecene mycotoxins are potent inhibitors of protein synthesis and injure organs with rapidly dividing cell populations, including the testis. Testicular structure and function were studied in male Lewis rats 1, 3, 7, 30, 60, and 90 days after exposure at age 12 weeks to anguidine at 1.7 mg/kg body weight given by ip injection. The dose was equivalent to 75% of the ip LD_{50}. Anguidine caused a gradual decline in testicular weight beginning 30 days after treatment. Sperm production was also reduced by 30 days, and the frequency of hypocellular seminiferous tubules increased by day 60. There was no evidence of recovery by 90 days. These changes are consistent with injury to proliferating cells early in the maturation sequence. Epididymal sperm reserves were reduced by 3 days after anguidine administration, prior to the reduction in sperm production, suggesting premature release of spermatozoa from the epididymis.

Key Words: trichothecene; anguidine; diacetoxyscirpenol; rats; reproduction; testis; epididymis; spermatozoa.

INTRODUCTION

Anguidine (diacetoxyscirpenol, DAS) is a tetracyclic sesquiterpenoid compound produced by at least 8 different fungi of the genus *Fusarium* and is classified as a trichothecene mycotoxin (1–3). Trichothecenes are potent inhibitors of protein synthesis, have been associated with naturally occurring outbreaks of mycotoxicosis in people and animals, and have been identified as components of chemical warfare agents (4–9). Exposure to trichothecenes causes necrosis of skin, lymphohematopoietic organs, and intestinal epithelium. Damage to these organs is reflected clinically by diarrhea and leukopenia, which may result in death (1,3,10). The cytotoxicity of the trichothecenes towards rapidly dividing cell populations in normal organs and to tumor models (11–14) led to the phase 1 and phase 2 evaluation of anguidine as an antineoplastic agent (15–20).

The testis was identified as a target of trichothecenes,with reports of aspermia in goats exposed to toxins derived from *F nivale* and cell degeneration in the testis of horses exposed to T-2 (1). Recently, we demonstrated that a single exposure to anguidine caused testicular degeneration in mice and that damage progressed in severity during the two weeks that the animals were studied after exposure (10). In contrast to this progressive degeneration in the testis, other anguidine-sensitive organs in the mouse recovered within days of exposure. The purpose of this experiment was to evaluate the effects of anguidine on testicular structure and function in the rat, a species for which there is considerable published information regarding sperm production and testicular morphology.

This study was supported in part by Contract DAMD 17-82-C-2235 from the U.S. Army Medical Research and Development Command and by the Pharmaceutical Manufacturers Association Foundation.

Address correspondence to: Dr. Michael W. Conner, Department of Safety Assessment, Merck Sharpe & Dohme Research Laboratories, West Point, PA 19486.

Received 11 *August* 1988; *Revision received* 12 *October* 1989; *Accepted* 23 *October* 1989.

MATERIALS AND METHODS

Animals

Male Lewis rats (Charles River Breeding Laboratories, Wilmington, MA), age 11 weeks, were housed individually in suspended clear polycarbonate tubs on corn cob bedding (Bed-O-Cobs, Anderson's Cob Division, Maume, OH) in a biohazards containment suite with a 12 h:12h light:dark cycle at 20 to 22 °C, 40 to 50% relative humidity, for 1 week prior to anguidine exposure. Food (Agway Pro 3000, Agway, Rahway, NJ) and distilled water were offered for ad libitum consumption. Anguidine was given at 12 weeks of age, when sperm production is maximal in rats (21). Animals were weighed weekly and at time of sacrifice. All procedures involving animals were approved by the institutional animal care and use committee.

Anguidine

The anguidine used in these experiments, kindly provided by Dr. Thomas Strike of the National Cancer Institute (Bethesda, MD), was purified by crystallization

from hexane–ether, dissolved in 10% aqueous dimethylsulfoxide (DMSO, Burdick and Jackson Laboratories, Inc., Muskegan, MI) at a concentration of 2 mg/mL and administered by intraperitoneal injection (ip) at 1.7 mg/kg body weight. This exposure is equivalent to 75% of the ip LD_{50} in Lewis rats as measured in this laboratory. Control rats were given an equivalent volume of 10% aqueous DMSO.

Experimental design

The number of spermatids in the testis, daily sperm production, epididymal sperm reserves, transit time for sperm through the epididymis, and morphology of the testis and epididymis were evaluated in 5 rats from each treatment group 1, 3, 7, 30, 60, and 90 days after anguidine administration according to procedures described below. Observations were extended to 90 days in order to include 6 cycles of the seminiferous epithelium (78 days in the rat), as might be required to detect injury if damage were limited to spermatogonia (22).

At the time of sacrifice, rats were anesthetized by intramuscular injection of 40 mg/kg of ketamine (Ketaset, Bristol Laboratories, Syracuse, NY) and 10 mg/kg xylazine (Rompun, Haver Lockhart, Shawnee, KS). The left testis and epididymis were exposed through a scrotal incision and removed after ligation of the testicular vessels. Spermatids and spermatozoa were enumerated in these organs using techniques described below. The right testis was fixed for morphologic evaluation by whole-body vascular perfusion with fixative. In this procedure, the thorax was opened and a polyethylene catheter (Intramedic, PE 240, Clay Adams, Parsippany, NJ) was passed through the left ventricle into the aorta and secured by a ligature. The right atrium was incised and the vascular system perfused at 100 cm H_2O with heparinized saline until the fluid from the right atrium was clear (approximately 2 min) and then with 3% glutaraldehyde in 0.1 M cacodylate buffer for 5 min. The right testis and epididymis were removed, stored in fixative for an additional 2 h, and processed for morphologic examination as described below.

Enumeration of spermatids and spermatozoa

The left testis and epididymis were separated, dissected free from fat, and weighed. The testis was minced in 25 mL cold (4 °C) 0.15 M NaCl containing 0.01% Triton X-100 and homogenized for 1 min (Polytron, Brinkmann Instruments, Westbury, NY, operated at setting 5). Homogenization-resistant spermatids in a 1:4 dilution of homogenate were counted in duplicate in a hemocytometer. The epididymis was prepared and the spermatozoa enumerated in a similar fashion except that the period of homogenization was extended to 1.5 min. The spermatid and spermatozoa concentrations in the homogenates were used to calculate the number of homogenization-resistant spermatids per testis, the number of homogenization-resistant spermatids per g testis, and the epididymal sperm reserves. The daily sperm production by the testis was calculated by dividing the number of spermatids per testis by 6.1, according to the method of Robb et al. (21). Epididymal transit time was calculated by dividing the epididymal sperm reserves by the daily sperm production (21).

Morphologic evaluation

The right testis and epididymis were prepared for microscopy by embedding a transverse section of the right testis and sections of the head and tail of the epididymis in glycolmethacrylate, sectioning at 1.5 μm and staining with the periodic acid-Schiff technique followed by a counterstain of Meyer's Harris hematoxylin (23). Forty (40) round or nearly round tubule profiles from each testis were selected at random and classified as Stage I through XIV according to the system of Leblond and Clermont (24). Tubules with morphologic abnormalities were tabulated according to the type of abnormality (e.g., retention of step 19 spermatids beyond Stage VIII, hypocellularity, presence of multinucleated spermatids). Because 40 tubules per testis was expected to be an insufficient sample size for describing the distribution of 14 tubule stages in individual animals, the data from the 5 animals from each group at each observation time were pooled and the frequency of stages expressed as a percent of the 200 tubules examined per group. The minor diameter of 30 randomly selected round or nearly round tubule profiles was measured on a video display that was calibrated using a stage micrometer. Thirty tubules is in excess of the number of tubules required to obtain a reliable estimate of the average tubule diameter in the rat testis (25).

Statistical analysis

Effects of anguidine treatment on body and organ weights and counts of spermatids and spermatozoa were analyzed by two-way analysis of variance (ANOVA). If the analysis of variance demonstrated a group effect, the data at individual observation times were compared with the Student's t test. Tubule classification data were analyzed by the chi-squared technique. All statistical analyses were performed with commercially available software (PC Statistician and PC ANOVA, Human Systems Dynamics, Northridge, CA). The level of significance was chosen at $P < 0.05$.

RESULTS

The first 3 to 7 days following anguidine exposure were characterized by decreases of 12 to 13% in body

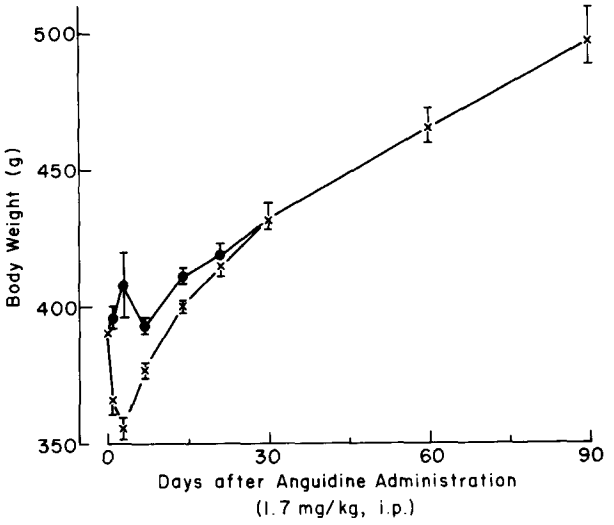


Fig. 1. Effect of anguidine on body weight of Lewis rats. Body weight was decreased on 3, 7, and 14 days after anguidine administration ($P < 0.05$, Student's t test). Bars represent standard errors.

weight ($P < 0.05$) (Figure 1) and left testis weight ($P < 0.05$) (Figure 2). Body weights of the anguidine-treated rats returned to control values by 30 days and were no different from controls at later time periods. In contrast, the left testis weights of anguidine-treated rats were similar to controls at 30 days but were lower at 60 and 90 days. At 90 days the left testis of anguidine-treated rats was only 73% of the weight of controls.

Spermatid content and sperm production in the left testis are represented on the same graph (Figure 3) using double axes since these parameters are related by a

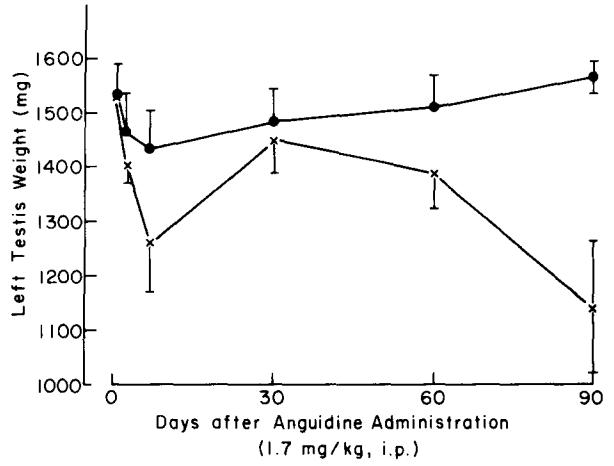


Fig. 2. Effect of anguidine on left testis weight in Lewis rats. The left testis weight was lower in anguidine-treated rats on days 3, 7, 60, and 90 following anguidine administration ($P < 0.05$, Student's t test). Bars represent standard errors.

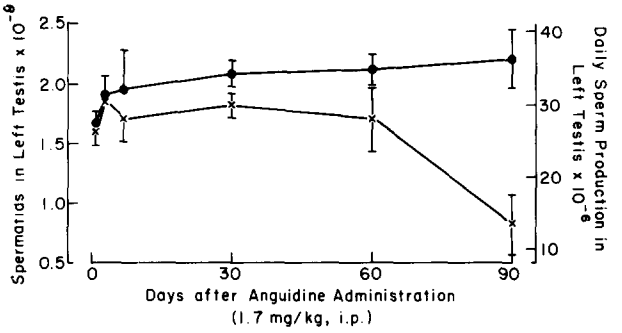


Fig. 3. Spermatids in the left testis of Lewis rats given anguidine. The number of spermatids was decreased in the left testis 30, 60, and 90 days following anguidine treatment ($P < 0.05$, Student's t test). Bars represent standard errors.

constant factor (6.1). These parameters were decreased ($P < 0.05$) by 30 days after anguidine exposure. At 90 days spermatid content and sperm production in anguidine-treated animals was only 37% of control values. These results were not appreciably altered by expressing the data as spermatids per g testis or sperm production per g testis to normalize for the loss of testicular weight at the later observation times (Figure 4). Spermatozoa were decreased in the epididymis 3, 7, 30, and 60 days after exposure to anguidine (Table 1). The decrease in epididymal sperm reserves on days 3, 7, and 30, without a significant decrease in sperm production, resulted in a lower calculated sperm transit time in the epididymis.

Table 2 shows the percentage of tubules classified as Stages I through XIV and the frequency of the most commonly observed abnormalities. Since chi-squared analysis indicated that anguidine treatment had no effect on the distribution of tubules among stages I through XIV, for those tubules that met the criteria for these

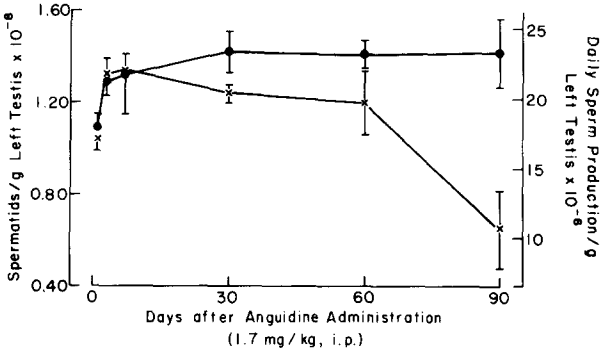


Fig. 4. Spermatids per gram of left testis of rats given anguidine. The number spermatids/g of left testis was lower 30, 60, and 90 days following anguidine treatment ($P < 0.05$, Student's t test). Bars represent standard errors.

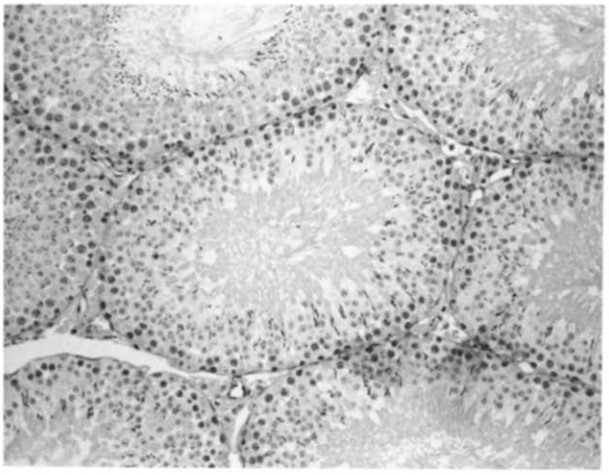
Table 1. Effect of anguidine on number of spermatozoa and sperm transit time in left epididymis[a]

Days after treatment	Spermatozoa (10^8)		Transit time (days)	
	DMSO	Anguidine	DMSO	Anguidine
1	2.26 ± 0.10	2.16 ± 0.20	8.2 ± 0.5	8.2 ± 0.8
3	2.91 ± 0.23	2.19 ± 0.06[b]	9.3 ± 0.3	7.2 ± 0.4[b]
7	2.72 ± 0.26	1.04 ± 0.17[b]	8.8 ± 0.8	3.7 ± 0.4[b]
30	3.07 ± 0.18	1.52 ± 0.22[b]	8.8 ± 0.9	5.1 ± 0.7[b]
60	3.12 ± 0.17	2.14 ± 0.30[b]	9.0 ± 0.7	7.8 ± 1.0
90	1.92 ± 0.35	1.20 ± 0.36	7.8 ± 1.0	7.9 ± 1.3

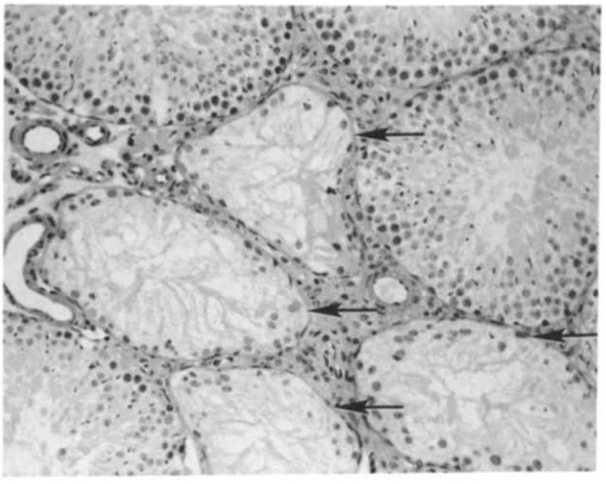
[a]Results are expressed as mean ± SE.
[b]$P < 0.05$ compared with DMSO, t test.

stages, the data are presented in a simplified format, combining stages I through XIV. The most common morphologic abnormality observed in seminiferous tubules of anguidine-treated rats was decreased cellularity. Hypocellular tubules (Figure 5) had few or no germinal epithelial cells and consisted almost entirely of Sertoli cells which often contained vacuoles (Figure 5). There was a notable background incidence of hypocellular tubules in testes of controls (0 to 7%), but the frequency was markedly higher in anguidine-treated rats at 60 (27.5%) and 90 days (47%). The visual impression that decreased cellularity was associated with smaller tubules was confirmed by measuring average tubule diameters ($P < 0.05$, two-way ANOVA, Table 3).

Also observed in testes of anguidine-treated rats were multinucleated giant spermatids (Figure 6) and inhibited release of Step 19 spermatids (Figure 7). Multinucleated giant spermatids were first noted 7 days after anguidine administration, were most frequent at 90 days (5.5%), and were not observed in testes of control rats. Inhibited release of step 19 spermatids, characterized by the presence of these spermatids beyond Stage VIII, was most frequent 7 days after anguidine treat-



(a)



(b)

Fig. 5. (a) Normal seminiferous tubules in a Lewis rat 90 days after treatment with DMSO (× 104). (b) Hypocellular seminiferous tubules (arrows) in Lewis rat 90 days after anguidine administration (1.7 mg/kg, ip). Note depletion of spermatogonia, spermatocytes, and spermatids (× 104).

Table 2. Frequency (%) of normal tubules and common morphologic abnormalities in seminiferous tubules of rats treated with anguidine or DMSO[a]

Days after treatment	Normal (Stages I through XIV) tubules		Hypocellular tubules		Multinucleate round spermatids		Inhibited release of Step 19 spermataids	
	DMSO	Anguidine	DMSO	Anguidine	DMSO	Anguidine	DMSO	Anguidine
1	95.0	98.0	4.5	0.0	0.0	0.0	0.0	0.0
3	94.0	100.0	4.5	0.0	0.0	0.0	0.0	0.0
7	89.0	76.0	7.0	14.0[b]	0.0	1.5	0.0	4.5[b]
30	100.0	90.0	0	3.0	0.0	0.0	0.0	0.5
60	98.0	68.0[b]	2.0	27.5[b]	0.0	2.0	0.5	1.0
90	95.0	46.0[b]	4.5	47.0[b]	0.0	5.5[b]	0.0	0.0

[a]Data are expressed as percent of 200 tubules examined (40 tubules/testis, 5 testes/group at each observation time.
[b]$P < 0.05$ compared with DMSO, chi-squared.

Table 3. Effect of anguidine on diameter (μm) of seminiferous tubules of the right testis[a]

Days after treatment	DMSO	Anguidine
1	303 ± 8	319 ± 11
3	292 ± 11	293 ± 4
7	307 ± 11	271 ± 26
30	295 ± 7	273 ± 8
60	327 ± 14	284 ± 24
90	289 ± 14	245 ± 26

[a]Data are expressed as mean ± SE. Anguidine treatment decreased the diameter of the seminiferous tubules ($P < 0.05$, two-way ANOVA).

ment, representing 4.5% of the tubules examined. Inhibited release of spermatids was a rare observation in testes from control rats (1/1200 tubules examined).

Anguidine-induced morphologic changes in the epididymis included distension of the interstitial space, consistent with interstitial edema (Figure 8), and decreased spermatozoa in tubules. The distension of the interstitial space was most pronounced 3 days after anguidine administration, corresponding to the time when epididymal weight was maximal (Table 4). Many of the epididymal tubules at this time contained few mature spermatozoa, in contrast to the tubules of controls (Figure 8). Large, round, multinucleated spermatids were present in some epididymal tubules of treated rats (Figure 9). At 60 and 90 days the morphologic changes were accompanied by decreased epididymal weights.

DISCUSSION

These results demonstrate that the testis of Lewis rats is sensitive to anguidine and that there is a relatively long period between exposure and changes in sperm production. The decreased sperm production was associated with an increase in the frequency of hypocellular seminiferous tubules. The presence of morphologic abnormalities in the testis weeks after exposure, at times when other anguidine-sensitive organs have returned to normal (26) is similar to the effects of anguidine on the mouse testis (10). There are, however, important differences in the testicular response between these two species. In the mouse, morphologic changes develop within 24 h, and significant numbers of hypocellular tubules are present 2 weeks after administration of anguidine at 75% of the ip LD_{50} (10). In contrast, the appearance of hypocellular tubules in the Lewis rat is delayed, with the number of hypocellular tubules not increasing until 60 days after exposure.

The difference in the rate of lesion progression between the testis and other anguidine-sensitive organs may be explained, in part, on the basis of cell turnover time. Generally, the interval from exposure to agents that damage mitotically active cells (e.g., radiation or cytotoxic drugs) to organ failure correlates with the turnover time of differentiated cells (27). Damage to the intestine by radiation or cytotoxic drugs, for example, is characterized by loss of surface epithelium within 4 to 7 days. This interval corresponds to the normal rate of progression of cells along the basement membrane towards the tip of the villus. An organ with a longer cell turnover time, the lung, has a correspondingly longer interval (85 to 150 days) between exposure to radiation and the development of pneumonitis. The time required for dividing spermatogonia to mature and be released

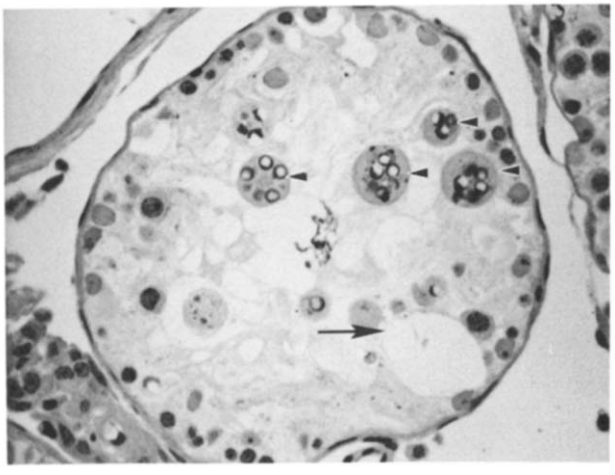


Fig. 6. Photomicrograph of seminiferous tubule with multinucleated round spermatids (arrowheads) and a vacuolated Sertoli cell (arrow) in a Lewis rat 90 days after treatment with anguidine (×260).

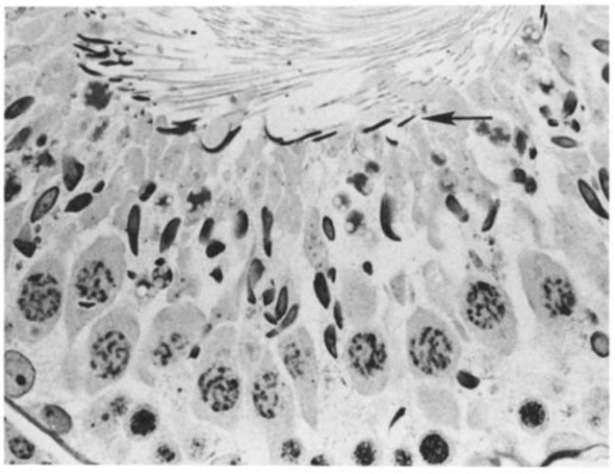
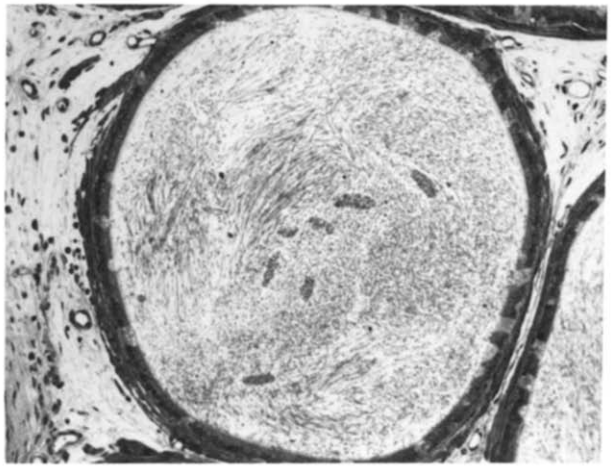
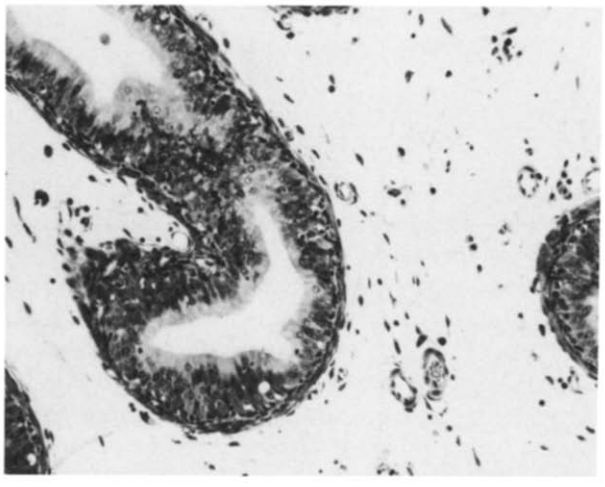


Fig. 7. Inhibited release of Step 19 spermatids (layer of spermatids nearest to tubule lumen, arrow) in testis of Lewis rat 7 days after administration of anguidine (×416).



(a)



(b)

Fig. 8. (a) Photomicrograph of normal tubules from the tail of the epididymis of a Lewis rat 3 days after treatment with DMSO. The tubules contain many spermatozoa and are closely apposed (× 104). (b) Photomicrograph of a tubule from the tail of the epididymis of a Lewis rat 3 days after treatment with anguidine. Note absence of spermatozoa in the lumen of the tubule. The distended space between tubules indicates the presence of excess interstitial fluid (edema) (× 104).

as Step 19 spermatids (4.5 cycles of the seminiferous epithelium) is approximately 58 days in the rat (22), and diminished sperm production, as an expression of spermatogonial injury, may not be evident for weeks after toxin exposure. The results of this experiment suggest, therefore, that cells in the rat testis with the greatest sensitivity to the cytotoxic effects of anguidine are early in the maturation sequence. The cycle of seminiferous epithelium in the mouse is shorter than in the rat (8.9 compared with 12.9 days), and it would be expected that spermatogonial injury would be evident somewhat earlier in this species (22), but not as early as 2 weeks. The presence of hypocellular tubules in the mouse just two

Table 4. Effect of anguidine on weight (mg) of left epididymis[a]

Days after treatment	DMSO	Anguidine
1	552 ± 10	594 ± 16
3	550 ± 14	643 ± 21[b]
7	563 ± 44	590 ± 42
30	612 ± 14	550 ± 31
60	650 ± 20	576 ± 16[b]
90	663 ± 17	574 ± 25[b]

[a]Results are expressed as mean ± SE.
[b]$P < 0.05$ compared with DMSO, t test.

weeks after anguidine administration (10) suggests, therefore, that there is a greater sensitivity to anguidine of cells later in the maturation sequence in this species.

An expected result of diminished sperm production and atrophy of seminiferous tubules is a decrease in the weight of the testis (22). This has been demonstrated for a number of testicular toxins (28–30). An association between decreased sperm production and loss of testicular weight is confirmed in this study by the similar magnitude of diminution of both of these parameters at 60 and 90 days. The transient decrease in testicular weight at 3 and 7 days, however, is not associated with changes in sperm production or cellularity of seminiferous tubules. Thus, while decreased sperm production is expected to result in a decrease in testicular weight, testicular weight may decrease without a corresponding decrease in sperm production. The basis for the transient decrease in testicular weight could not be established from the observations made in this study. As this early,

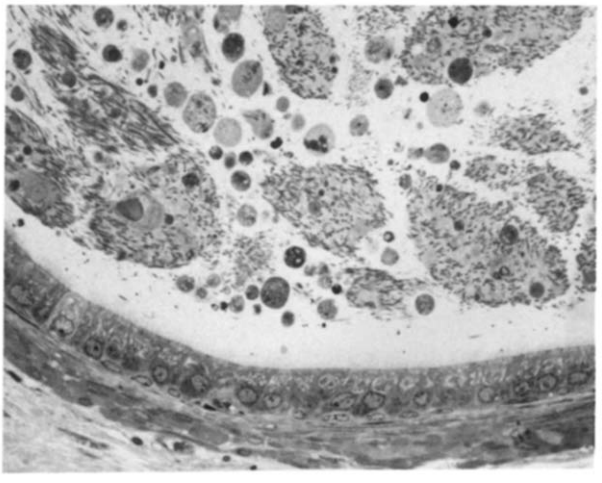


Fig. 9. Photomicrograph of a tubule from the tail of the epididymis of a Lewis rat 60 days after treatment with anguidine. The tubule contains many multinucleated round spermatids (× 260).

transient decrease in testicular weight corresponds to the period of decreased body weight following anguidine administration, it is possible that the decrease in testicular weight is related to the overall loss of body mass during this period.

Inhibited spermiation (retention of Step 19 spermatids beyond Stage VIII) was most frequent 7 days following anguidine administration. Inhibited spermiation has been described in rats exposed to a number of toxins including methyl chloride (31,32), glycol monomethyl ether (33), and dibutyryl cyclic AMP (34). It has also been described in rats fed a vitamin-A-deficient diet (35). The mechanisms responsible for the inhibited spermiation in these instances have not been fully elucidated. Excess fluid surrounding retained Step 19 spermatids in rats treated with dibutyryl cyclic AMP and association of the retained spermatids with masses resembling residual bodies in rats given methyl chloride have been cited as evidence for interruption of the tubulobulbar complexes as a possible mechanism for the retention of the Step 19 spermatids (33,34). Neither of these changes were observed in this study and the basis of the inhibited spermiation remains unknown. The presence of inhibited spermiation in this study does reflect sensitivity to anguidine of cells other than germinal cells early in the maturation sequence.

The significance of the Sertoli cell vacuolization is difficult to establish on the basis of the data available in this study. Vacuoles were most evident in Sertoli cells in hypocellular tubules and may, therefore, merely reflect loss of germinal epithelium from these spaces. The possibility that Sertoli cells are also targets of anguidine must also be considered. Similar vacuoles are evident in published photomicrographs of seminiferous tubules from rats exposed to a number of testicular toxins, including methyl chloride (32) and glycol monomethyl ether (33). These latter toxins and anguidine also inhibit spermiation, raising the possibility that these phenomena are related.

The decrease in epididymal sperm reserves by day 3, prior to the decrease in sperm production, indicates that anguidine altered epididymal transit time. The epididymal transit times reported here are calculated from the daily sperm production and the epididymal sperm reserves and do not, therefore, provide an independent measure of transit time. These values do, however, indicate the magnitude of the effect of anguidine on transit time, with transit times in the anguidine-treated rats falling to approximately 50% of control values by 7 days. This was a short-term effect, with transit times returning to normal by 60 days. This suggests that the reduction in transit time at earlier time points is related to the acute effects of anguidine on either the epididymis or spermatozoa within the epididymis. Interpretation of sperm transit times and epididymal sperm reserves at 90 days is impeded by the decrease in epididymal sperm on both control and anguidine-treated rats at this time. The basis for the decrease in control values at this time is not apparent, but is likely related to individual variability. Despite this variation in the control values, the trend observed at earlier observation times, that anguidine decreased epididymal sperm reserves, is still apparent at 90 days.

The hypothesis that changes in epididymal function may play a role in the altered transit time is strengthened by the temporal coincidence of interstitial edema of the epididymis and decreased sperm transit times on day 3. The edema was of sufficient severity on day 3 that the epididymal weights were increased even in the face of a decrease in sperm content. Altered epididymal function has also been proposed as the basis for reductions in epididymal sperm reserves that have been observed after treatment with cyclophosphamide (36). As in this study, the reduction in epididymal sperm reserves after cyclophosphamide occurred prior to a decrease in sperm production. Since epididymal sperm were not collected for direct examination of morphology or mobility, it is not possible to establish whether anguidine had any direct effects on spermatozoa that might also have contributed to the decrease in transit time.

The most profound effects of anguidine on the testis of Lewis rats, decreased sperm production and the increase in hypocellular tubules, reflect injury to germinal cells early in the maturation sequence. Evidence provided by this study that there are a number of other targets of anguidine in the reproductive tract includes inhibited spermiation, acute alterations in epididymal transit times, and vacuolization of Sertoli cells. Further studies are required to establish to what degree these phenomena are dose or strain dependent.

REFERENCES

1. Ueno Y. Trichothecene mycotoxins: Mycology chemistry and toxicology. Adv Nutr Res. 1980;3:301–53.
2. Carlton WW, Szczech GM. Mycotoxicosis in laboratory animals. In: Wyllie TD, Morehouse LG, eds. Mycotoxic fungi mycotoxins and mycotoxicoses. New York: Marcel Dekker; 1978:333–462.
3. Doyle TW, Bradner WT. Trichothecanes. In: Anticancer agents based on natural product models. Orlando, Florida: Academic Press; 1980:43–72.
4. Ueno Y. Mode of action of trichothecenes. Pure Appl Chem. 1977;49:1737–45.
5. Watson SA, Mirocha CJ, Hayes AW. Analysis for trichothecenes in samples from Southeast Asia associated with yellow rain. Fundam Appl Toxicol. 1984;4:700–17.
6. Rosen RT, Rosen JD. Presence of four fusarium mycotoxins and synthetic material in yellow rain. Evidence for the use of chemical weapons in Laos. Biomed Mass Spectrom. 1982;9:443–50.
7. Holden C. Unequivocal evidence of Soviet toxin use. Science. 1982;216:154–5.
8. Mirocha CJ, Pawlosky RA, Chatterjee K, Watson S, Hayes W. Analysis for Fusarium toxins in various samples implicated in biological warfare in Southeast Asia. J Assoc Off Anal Chem. 1983;66:1485–1499.
9. Spyker MS, Spyker DA. Yellow rain: chemical warfare in

Southeast Asia and Afghanistan. Vet Hum Toxicol. 1983;25: 335–40.
10. Conner MW, deCamargo J, Punyarit P, Riengropitak S, Rogers AE, Newberne PM. Toxicity of anguidine in mice. Fundam Appl Toxicol. 1986; 7:153–64.
11. Corbett TH, Griswold DP, Roberts BJ, Peckham JC, Schabel FM. Evaluation of single agents and combination of chemotherapeutic agents in mouse colon carcinomas. Cancer. 1977;40:2660–80.
12. Hill BT. Preclinical screening for drugs effective against 5-fluorouracil-resistant cell lines with a murine LS178Y cell line in vitro. J Natl Cancer Inst. 1983;71:335–40.
13. Hromas R. Barlogie B, Schwartzendruber D, Drewinko B. Selective protection by anguidine of normal vs. transformed cells against 1-beta-*O*-arabinofuranosylcytosine and adriamycin. Cancer Res. 1983;43:1135–7.
14. Rosencweig M, VonHoff DD, Staquet MJ, Schein PS, Penta JS, Goldin A, Muggia FM, Freireich EJ, DeVita VT. Animal toxicology for early clinical trials with anticancer agents. Cancer Clin Trials. 1981;4:21–8.
15. Adler SS, Lowenbraun S, Birch B, Jarrell R, Garrard J. Anguidine, a broad phase II study of southeastern cancer study group. Cancer Treat Rep. 1984;68:423–5.
16. Bukowski R, Vaughn C, Bottomley R, Chen T. Phase II study of anguidine in gastrointestinal malignancies: a southwest oncology group study. Cancer Treat Rep. 1982;66:381–3.
17. Goodwin JW, Haas CD, Fabian C, Heller–Bettinger I, Hoogstraten B. Phase I evaluation of anguidine (diacetoxyscirpenol, NSC-141537). Cancer. 1978;42:23–6.
18. Goodwin JW, Stephens R, McCracken JD, Groppe C. Therapy for advanced colorectal cancer with a combination of 5-FU and anguidine: A southwest oncology group study. Cancer Treat Rep. 1981;65:359.
19. Goodwin JW, Bottomley RH, Vaughn CB, Frank J, Pugh RP. Phase II evaluation of anguidine in central nervous system tumors: A southwest oncology group study. Cancer Treat Rep. 1983;67: 285–6.
20. Murphy WK, Burgess MA, Valdiviesco M, Livingston RB, Bodey GP, Freireich EJ. Phase I clinical evaluation of anguidine. Cancer Treat Rep. 1978;62:1497–502.
21. Robb GW, Amann RP, Killian GJ. Daily sperm production and epididymal reserves of pubertal and adult rats. J Reprod Fertil. 1978;54:103–7.
22. Amann RP. Use of animal models for detecting specific alterations in reproduction. Fundam Appl Toxicol. 1982;2:13–26.
23. Chapin RE, White RD, Morgan KT, Bus JS. Studies of lesions induced in the testis and epididymis of F-344 rats by inhaled methyl chloride. Toxicol Appl Pharmacol. 1984;76:328–43.
24. Leblond CP, Clermont Y. Definition of the stages of the cycle of the seminiferous epithelium in the rat. Ann NY Acad Sci. 1952;55:548–73.
25. Berndtson WE, Neefus C, Foote RH, Amann RP. Optimal replication for histometric analyses of testicular function in rats or rabbits. Fundam Appl Toxicol. 1989;12:291–302.
26. Malarkey DE, Conner BH, Rogers AE, Conner MW, Newberne PM. Reduction of anguidine toxicity in rats by atropine and methylatropine. Fundam Appl Toxicol. 1989;97:512–24.
27. Denekamp J. Cell kinetics and radiation biology. Int J Radiat Biol. 1986;49:357–80.
28. Foote RH, Berndtson WE, Rounsaville TR. Use of quantitative testicular histology to assess the effect of dibromochloropropane (DBCP) on reproduction in rabbits. Fundam Appl Toxicol. 1986;6: 638–47.
29. Kluwe WM, Weber H, Greenwell A, Harrington F. Initial and residual toxicity following acute exposure of developing male rats to dibromochloropropane. Toxicol Appl Pharmacol. 1985;79: 54–68.
30. Evenson DP, Baer RK, Jost LK, Gesch RW. Toxicity of thiotepa on mouse spermatogenesis as determined by dual-parameter flow cytometry. Toxicol Appl Pharmacol. 1986;82:151–63.
31. Chellman GJ, Hurtt ME, Bus JS, Working PK. Role of testicular versus epididymal toxicity in the cytotoxic damage in Fischer-344 rat sperm by methyl chloride. Reprod Toxicol. 1987;1:25–35.
32. Chapin RE, White RD, Morgan KT, Bus JS. Studies of lesions induced in the testis and epididymis of F-344 rats by inhaled methyl chloride. Toxicol Appl Pharmacol. 1984;76:328–43.
33. Chapin RE, Dutton SL, Ross MD, Swaisgood RR, Lamb JC. The recovery of the testis over 8 weeks after short-term dosing with ethylene glycol monomethyl ether: histology, cell specific enzymes and rete testis fluid protein. Fundam Appl Toxicol. 1985;5: 515–25.
34. Gravis CJ. Ultrastructural observations on spermatozoa retained within the seminiferous epithelium after treatment with dibutyryl cyclic AMP. Tissue Cell. 1980;12:309–22.
35. Huang HF, Marshall GR. Failure of spermatid release under various vitamin A states — an indication of delayed spermiation. Biol Reprod. 1983;28:1163–72.
36. de la Calle JFV, de Queiroz F, Garnier DH, Kercret H, Folliot R, Jegou B. Reproductive effects of the anticancer drug cyclophosphamide in male rats at different ages. Arch Androl. 1989;22: 251–63.