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Regular Paper

A study Assessing the Genotoxicity in Rats after Chronic Oral Exposure to a Low Dose of Depleted Uranium

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Depleted uranium/Ingestion/Genotoxicity/Micronuclei/Comet assay.

Purpose: The aim of this study was to evaluate the potential genotoxicity induced by chronic oral exposure to depleted uranium (DU). **Materials and methods:** Weanling Wistar rats (F_0), 50/sex/group, were exposed to DU in food at doses of 0, 4, or 40 mg $kg^{-1}day^{-1}$ for four months. They were subsequently mated, resulting in the birth of F_1 rats. Fifty F_1 weanlings/sex/group were exposed for four months to the same dose levels as their parents. After four months, the uranium content in the tissues, the potential damage to the genetic material, and pathomorphological changes of the testicles were observed in both F_0 and F_1 rats. The genotoxicity of DU was evaluated by the following methods: sperm abnormality assessment, the bone-marrow micronucleus test, and the comet assay. **Results:** Uranium content in F_1 rats was significantly higher than that in F_0 rats in both the kidney and ovary ($p < 0.05$). The sperm abnormality rate, marrow cell micronuclei rate, comet tail length, and tailed cell percentage increased in each treatment group in each generation compared with the control group ($p < 0.05$). When comparing F_1 with F_0 rats, significant differences were detected for most of the indicators, with F_1 rats always exhibiting more damage ($p < 0.05$). With regard to pathomorphological changes in the testicles, the sperm displayed atypical changes, including thickening of the anachromasis nucleolus, which seemed to be more severe in F_1 rats. **Conclusion:** Genotoxicity may be induced in rats after chronic oral exposure to a low dose of DU.

INTRODUCTION

Depleted uranium (DU) is the by-product generated when uranium is processed into nuclear fuel. In DU, most of the U^{235} and U^{234} isotopes have been selectively removed through industrial processes, meaning that the radiological hazard of DU is less than that from natural or enriched uranium.[1)] The particles have a long penetrating ability, and they are hazardous only if the uranium is ingested or inhaled.[2)]

A large number of DU weapons have been used since the Gulf War began in 1991, resulting in large-scale environmental pollution.[3,4)] A number of soldiers who participated in that war have claimed to be suffering from a new chronic illness generally known as Gulf War Syndrome (GWS), a poorly understood disease with multiple symptoms and many theories about its aetiology and pathogenesis.[4,5)] DU has been used as an armour-penetrating ammunition in the Balkans. It has been suggested that DU could also be related to a new illness, the Balkan syndrome, which is under investigation.[6)]

The potential health hazards associated with exposure to DU alloys are both radiological and chemical,[7,8)] and both modes of toxicity would be expected to occur in cases where DU projectiles or fragments become internally deposited (e.g., embedded) in tissue.[9)] Kidney and bone are known to represent the primary reservoirs for DU.[10)] DU exerts a nephrotoxic effect through its chemical action, which is localised primarily in the renal proximal tubules.[11,12)] Implantation of DU alloys into rat muscle has been shown to increase the frequency of implantation site soft tissue sarcomas,[13)] while in vitro studies have shown that DU is mutagenic[14)] and clastogenic,[15)] suggesting that embedded DU could be carcinogenic in humans. With respect to the reproductive effects of DU, Feugier and coworkers[16)] assessed the alterations of mouse oocyte quality after a sub-chronic exposure to DU. Four different DU concentrations were investigated: 0 (control), 10 (DU_{10}), 20 (DU_{20}), and 40 mgL^{-1} (DU_{40}). DU did not influence the intensity of ovulation, but affected oocyte quality. An abnormal perivitelline space ($p < 0.01$)

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or absence of the 1st polar body ($p < 0.01$) was identified as the main characteristic of DU impact. Only a short abstract regarding the maternal and/or developmental effects of DU could be found in the literature. Female rats were exposed to one of five doses (not reported) of DU via surgically implanted pellets and then bred with male rats. Dams were euthanized on gestation day 20. No adverse effects on maternal weight gain, food consumption, water intake, or kidney histology were observed, and parameters such as litter size, pup weight, and sex ratio were also not affected by DU exposure.[17] Although some results have suggested potential health hazards associated with exposure to DU caused by long-term ingestion of low-dose depleted uranium in the food and water, little information is currently available regarding its damage to the body.

The aim of this study was to observe the potential damage of depleted uranium on genetic material and pathomorphological changes of reproductive organs and to evaluate the effect of DU on genotoxicity. In the present study, the DU dose used for rat intake was determined based on literature reports about environmental pollution levels of DU.[18] We observed the uranium content in the tissues; the abnormality rate of rat sperm using a high-power microscope; and DNA damage by the comet assay, which is a rapid, sensitive, and inexpensive measurement method,[19] and the micronucleus test; along with assessment of DNA damage by agarose gel electrophoresis. Finally, another group of F_0 rats was sacrificed at ages 7, 14, and 20 months, while homologous F_1 rats were sacrificed at ages of 5, 10 and 15 months, and some organs were taken for pathological observation. This is a preliminary study on long-term hazards of low doses of DU.

MATERIALS AND METHODS

Animals

One hundred and fifty male and 150 female hybrid mice (Wistar rats) weaned at three weeks of age were obtained from the Institute of Daping Zoology (The Third Military Medical University, SCXK (Chongqing) 2002003, China). The mice were collectively housed in groups of three in plastic cages under controlled conditions with a 12-h/12-h light/dark cycle, a temperature of 22.7°C, and a relative humidity of 55%. Experiments began after a week of acclimatisation. Three-week-old mice weighing 35–55 g were randomly divided into three groups, with each group containing 50 male and 50 female rats. Two groups (DU_4, DU_{40}) were exposed to food contaminated with 4 or 40 mg U kg^{-1} day^{-1}, respectively, and were compared with the normal control group fed ordinary food. Food, distilled water intake, body weight, and health status were recorded daily. Animals were euthanized by cervical dislocation. The study was conducted in accordance with Chinese legislation regarding the care of animals used for experimental purposes.

Contamination

DU (specific activity of 1.24×10^4 Bq g^{-1}) was purchased from the China National Munitions Corporation, Beijing. The isotopic composition of DU was defined as the following mass proportions: ^{238}U: 99.75%, ^{235}U: 0.20%, and trace ^{234}U. Since uranyl ions $(UO_2)^{2+}$ are the most stable species of uranium in solution and are the form in which this element generally exists in mammalian body fluids, uranium was administered as uranyl nitrate.[3] For animal exposure, two different solutions were prepared in order to obtain two concentrations of uranium: 4 mg U kg^{-1} day^{-1} (the low-dose group) and 40 mg U kg^{-1} day^{-1} (the high-dose group).

DU oral contamination model

Suckling mouse was exposed to DU in food until the period of sexual maturity. Each group contained 50 male and 50 female rats. Adults were mated at 120 days of age (at sexual maturity) and placed in open metal grid mating cages until the moment of conception, or until day 21. The bottoms of the mating cages were checked, and the date of conception was recorded twice daily by two trained technicians who searched for evidence of mating (e.g. seminal plugs). The appearance of a seminal plug was considered as evidence of successful mating and a sign of pregnancy. If no evidence of mating was found for the mated pair by day 21, the male was returned to his home cage, and the mating was recorded as a "failure." Parental rats gave birth to the first generation (F_1), and F_1 rats underwent the same feeding procedure as the homologous parents (F_0).

Uranium analyses

Over the four months after ingestion of DU, uranium content was measured in the kidneys, testicles, blood, and urine. The uranium content was measured by ICPMS (Beijing feather lun technology Co., LTD, China). Tissues (25–400 mg or 0.5 ml) were digested by addition of 0.5 ml of concentrated nitric acid and heated to 140°C for 2 h. The acid volume was reduced to approximately 200 µl, at which time the temperature was reduced to 110°C and 0.5 ml of ultrapure hydrogen peroxide was added. Samples were heated for an additional 1 h to complete the digestion. Samples were cooled and diluted to 5 ml with plasma-grade water to reduce the acid concentration in samples to 3%. Diluted samples were filtered through 0.22-mm PTFE syringe filters (Millex, Millipore, Bedford, MA) prior to the analysis. The quantitation limit for the instrument was 0.002 ppb. Recovery was determined from tissues spiked with 0.1–10 ng U/g tissue prior to digestion and determined to be 94–111%.

Sperm abnormality evaluation

During the four months following ingestion of DU, the rats were sacrificed (including both the F_0 and F_1 generations). The bilateral epididymides were excised, and the cauda epididymal fluid was diluted. The sperm were stained

with eosin and mounted on a glass slide. Sperm were enumerated using a haemocytometer under a light microscope. Abnormal sperm (dicephaly, double tails, microcephalic, or megacephalic sperm, etc.) were recorded from a differential count of 5000 sperm per group in order to calculate the percentage of morphologically abnormal sperm.

Rat bone marrow micronucleus (micronuclei, MN) test

During the four months following ingestion of DU, the rats were sacrificed to obtain bilateral femurs. The bone marrow was flushed out from the femurs using 1 ml of RPMI 1640 and centrifuged at 1200 rpm for 10 min. The supernatant was discarded. Evenly spread bone marrow smears were stained using the May-Grunwald and Giemsa protocol. The number of micronuclei was observed with an oil immersion lens (100 times magnification). For each group, 5000 polychromatic erythrocytes (PCE) were scored to quantitate the frequency of PCE containing MN (MNPCE). Finally, the micronucleus rate (%) was calculated.

Single cell gel electrophoresis or comet assay to detect DNA damage

After the rats were sacrificed, bilateral epididymides were placed in 3 ml of PBS buffer, cut, and scattered to dissociate the sperm. The mixed sample was filtered through a nylon net to create a cell suspension, and PBS buffer was used to maintain a cell concentration of $2\sim5 \times 10^6$/ml. The comet assay was performed under alkaline conditions, according to the method of Singh[19] with slight modifications. Frosted microscope slides were covered with a thin layer of 0.5% normal melting agarose at about 45°C (dissolved in Ca^{2+} and Mg^{2+}-free PBS). A coverslip was placed on the slide to promote even and firm attachment. Upon solidification of the agarose, the coverslip was gently removed, and 30 µl of the sample suspension mixed with 75 µl of low-melting-point agarose (LMPA) was added at 37°C. The coverslip was replaced, and the agarose was again allowed to solidify for 10 min at 4°C. After subsequently removing the coverslip, the slides were incubated in the dark at 4°C in a cuvette containing lysis solution (2.5 M NaCl, 0.1 M EDTA, 0.01 M Tris, and 10 g/L N-lauroylsarcosine sodium adjusted to pH 10 with NaOH, to which 1% Triton X-100 and 10% DMSO were added immediately before use) for five hours. After five hours, the dry slides were placed in the electrophoresis solution (300 mM NaOH, 1 mM EDTA, pH 13.0) for 20 min, and electrophoresis was carried out for 30 min at 25 V (1.1 V/cm) and 300 mA. The slides were neutralised in 0.4 mmol/l Tris buffer (to pH 7.5 with HCl) for 15 min × 3 times and stained with 70 µl of EB. Slides were scored using Comet Score software, and 400 cells were analysed per group. Tail length, tailed cell percentage, and head DNA percentage were used to estimate the DNA damage.

Research methods used for pathomorphological changes

An additional set of F_0 rats was killed at ages of 7, 14, and 20 months, and homologous F_1 rats were killed at 5, 10, and 15 months of age. Testicles and ovaries were dissected and fixed with 10% formaldehyde. Hematoxylin-eosin staining was used to observe pathomorphological changes. Animals were divided into age groups based on the following criteria: early stage (4 to 7 months), middle stage (7 to 14 months), and late stage (14 to 20 months).

Statistical analyses

For the quantitative data, means and standard deviations were calculated, and statistical tests were performed as described below. All statistical tests were performed at the $p < 0.05$ level of significance. All data were analysed with the SPSS12.0, Excel, and Systat 10.2 software (Systat Sofware Inc., Richmond, CA). Systat 10.2 was mainly used to determine the normality of the distributions of continuous variables using probability plots, skew, and kurtosis, as well as the one-sample Kolmogorov-Smirnov test. All variables were analysed by One-way ANOVA after normal distribution analysis and rank case, and Tukey's HSD was used for comparisons between the groups.

RESULTS

Uranium content (Table 1)

Uranium accumulated mainly in the kidney, with a significant difference between the high-dose and low-dose groups after chronic oral exposure to a low dose of depleted uranium ($p < 0.05$). Furthermore, in the low-dose group, uranium content in the F_1 rats was significantly higher than in the F_0 rats ($p < 0.05$). In the blood and urine, uranium content in each treatment group and generation was higher than that in the normal control group ($p < 0.05$), but there were no differences between either the high-dose and low-dose groups or between the F_0 and F_1 rats ($p > 0.05$). Differential accumulation of uranium in DU-exposed groups compared with the normal control group was also found in the ovary, with significant differences observed between the high-dose and low-dose groups in the F_1 rats ($p < 0.05$). Comparing F_0 with F_1 in the same dose group, uranium content in F_1 rats was significantly higher than that in F_0 rats ($p < 0.05$).

Sperm abnormality rate (Table 2)

In F_0 and F_1 rats in both the low and high-dose groups, the sperm abnormality rate was significantly higher than that in the normal control group after chronic oral exposure to low doses of depleted uranium ($p < 0.05$), with the high-dose group exhibiting higher abnormality rates than the low-dose group. Comparison of F_0 with F_1 rats in the same dose group revealed that the sperm abnormality rate in F_1 rats was significantly higher than that in F_0 rats ($p < 0.05$). Rat sperm abnormality mainly appeared in the head and body, and the

Table 1. Uranium content (ng/g) in the rat tissues after exposure of 4 months

Treatment group	Number of samples	blood	urine	kidney	ovary
Control	10	4.10 ± 1.08	6.00 ± 0.95	145.53 ± 10.25	11.82 ± 5.73
F_0 DU_4	10	150.30 ± 37.41*	289.33 ± 58.39*	1788.48 ± 759.54*	237.28 ± 36.60*
F_0 DU_{40}	10	131.96 ± 38.14*	384.75 ± 99.03*	11333.38 ± 9398.19*⋆	218.56 ± 137.64*⋆
F_1 DU_4	10	132.21 ± 39.78*	362.00 ± 95.26*	5764.68 ± 3589.11*◆	343.38 ± 111.34*◆
F_1 DU_{40}	10	176.39 ± 84.27*	458.33 ± 74.06*	16102.32 ± 10798.26*⋆	1265.94 ± 480.42*⋆◆

Compared with the normal group, *p < 0. 05;
Compared with the low-dose group in each generation, ⋆p < 0. 05;
Compared with the F_0 rats in each dose, ◆p < 0. 05.

Table 2. The comparison of rat sperm abnormality rate

Treatment group	Number of samples	Number of cells observed	sperm abnormality rate (%)
Control	10	5000	2.16 ± 1.01
F_0 DU_4	10	5000	5.67 ± 0.58*
F_0 DU_{40}	10	5000	7.23 ± 2.07*⋆
F_1 DU_4	10	5000	8.95 ± 1.34*◆
F_1 DU_{40}	10	5000	11.37 ± 3.26*⋆◆

Compared with the normal group, *p < 0. 05;
Compared with the low-dose group in each generation, ⋆p < 0. 05;
Compared with the F_0 rats in each dose, ◆p < 0. 05.

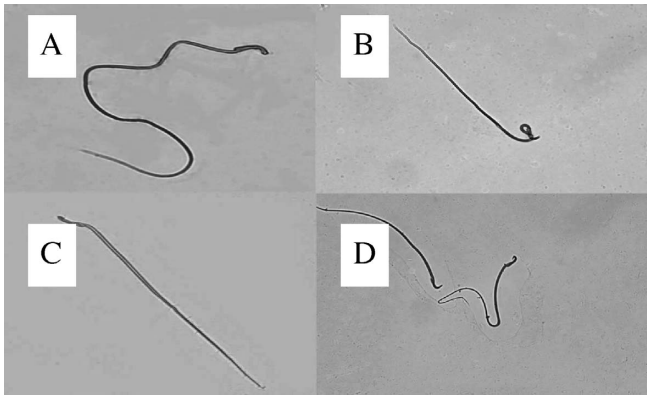

Fig. 1. Four kinds of rat sperm abnormalities. (A) Coiled tail with normal head: Coiled tail with a definite head shape especially accented by a marked hook. (B–D) Abnormal sperm heads as follows: (B) Amorphous head: Head hook does not have distinct shape but with a spherical spot at the tip; (C) No hook: The head is small and pin-head shaped, no hook; (D) Banana caput: Hook on head wrongly situated like a banana.

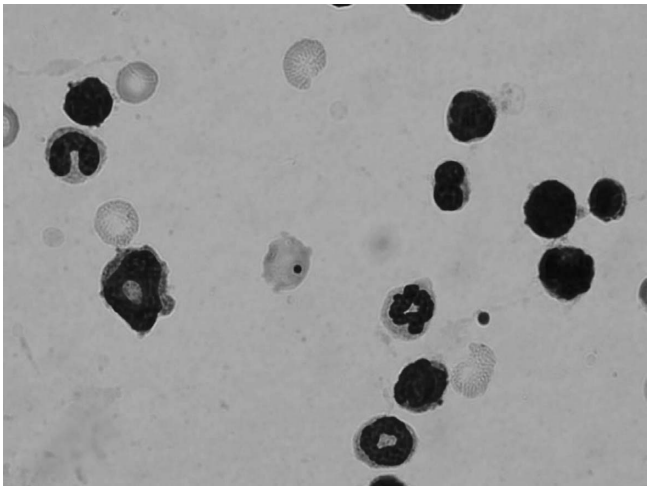

Fig. 2. The micronuclei in PCE of rat marrow (Giemsa dyeing, ×1000).

head abnormality mainly appeared hookless, amorphism, and banana caput (Fig. 1).

Bone marrow cell micronuclei rate (Table 3)

After chronic oral exposure to a low dose of depleted uranium, significant differences in sperm DNA damage were observed between the control group and the dose groups ($p < 0.05$), but in the low and high-dose groups, no significant differences were detected ($p > 0.05$). Comparing F_0 with F_1 rats in the same dose group, the micronuclei rate in F_1 rats was significantly higher than that in F_0 rats ($p < 0.05$). The results showing the micronuclei in the PCE of rat marrow, as visualised by Giemsa dyeing and acridine orange fluorescence staining, are presented in Fig. 2.

Table 3. The comparison of rat bone marrow cells micronuclei rate

Treatment group	Number of samples	PCE	MNPCE	micronuclei rate (%)
Control	15	5000	0.56 ± 0.22	0.9 ± 0.21
F_0 DU_4	15	5000	8.67 ± 1.98	8.96 ± 0.83*
F_0 DU_{40}	15	5000	9.10 ± 2.03	9.52 ± 1.39*
F_1 DU_4	15	5000	10.23 ± 3.16	11.40 ± 3.47*◆
F_1 DU_{40}	15	5000	13.15 ± 3.36	13.01 ± 2.65*◆

Compared with the normal group, *p < 0. 05;
Compared with the F_0 rats in each dose, ◆p < 0. 05.

Sperm DNA damage (Table 4)

After chronic oral exposure to a low dose of depleted uranium, significant differences in sperm DNA damage were observed between the control group and the dose groups ($p < 0.05$). Whether in F_0 or F_1 rats, the tailed cell percentage and tail length were higher than those obtained for the control group, while the head DNA percentage was lower than in the control group. Significant differences were also observed between the high and low-dose groups ($p < 0.05$). Comparison of F_0 with F_1 rats in the same dose group revealed that the tailed cell percentage and tail length in F_1 rats were significantly higher than those in F_0 rats ($p < 0.05$), while the head DNA percentage in F_1 rats was lower than that in F_0 rats ($p < 0.05$). Using the fluorescence microscope, comet sperm image analysis revealed the orange nucleus and broken DNA fragments resulting from injured cells, which migrated in the anode to form the phenomenon of tailing, like a comet.

Pathomorphological changes in the F_0 generation

The changes detected in the low-dose group were mainly present in the testicles/ovaries. In the early stage, the spermatogenic cell layer was reduced, and the stromal cells displayed mild proliferation. In the middle stage, the convoluted tubules were atrophied like a mesh, and there was an apparent reduction in the number of sperm, while stromal cells proliferated abnormally (Fig. 3, 4). In the late stage, the convoluted tubules were atrophied, the number of spermatogenic cells was decreased, and the stromal cells proliferated abnormally. The ovaries, however, exhibited no significant changes.

The changes detected in the high-dose group were mainly present in the testicles/ovaries. In the early stage, the spermatogenic cell layer and the number of sperm decreased, thickening of the pycnosis tube wall of the convoluted tubule was detected, and the stromal cells proliferated. In the middle stage, the convoluted tubules were atrophied like a mesh, larger nuclei and inconspicuous nucleoli were observed in the spermatogenic cells (atypical changes), and the stromal cells proliferated abnormally. In the late stage, the convoluted tubules were clearly atrophied and vacuolisation with malformation of the spermatogenic cells was apparent (larger nucleus and multinucleation), while stromal cells demonstrated strong proliferation (Fig. 5). With regard to the ovary, no significant anomalies were detected in the early stage, although the ovary was atrophied in the middle stage. In the late stage, the oocytes were vacuolar or mesh-like and demonstrated poor growth.

Pathomorphological changes in the F_1 generation

Changes in the low-dose group appeared mainly in the testicles/ovaries. In the early stage, some spermatogenic

Table 4. Analysis of sperm DNA damage in rats

Treatment group	Number of samples	Number of cells observed	Tailed cell percentage (%)	Tail length (ìm)	Head DNA percentage (%)
Control	15	400	3.81 ± 1.04	45.12 ± 10.23	87.32 ± 4.89
F_0 DU_4	15	400	6.75 ± 2.52*	89.17 ± 14.36*	75.73 ± 7.84*
F_0 DU_{40}	15	400	10.27 ± 3.64*★	133.5 ± 25.63*★	64.91 ± 6.23*★
F_1 DU_4	15	400	8.93 ± 2.85*◆	150.72 ± 35.47*◆	57.48 ± 9.45*◆
F_1 DU_{40}	15	400	15.47 ± 6.21*★◆	170.84 ± 45.8*★◆	40.45 ± 10.32*★◆

Compared with the normal group, *p < 0. 05;
Compared with the low-dose group in each generation, ★p < 0. 05;
Compared with the F_0 rats in each dose, ◆p < 0. 05.

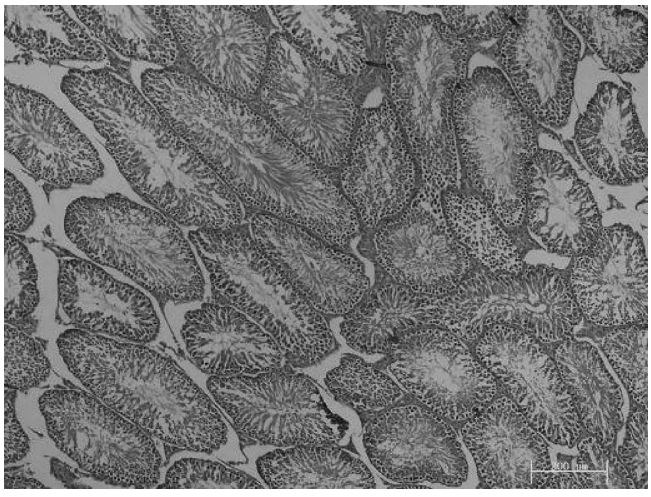

Fig. 3. Testis biopsy of the normal control rats (×100).

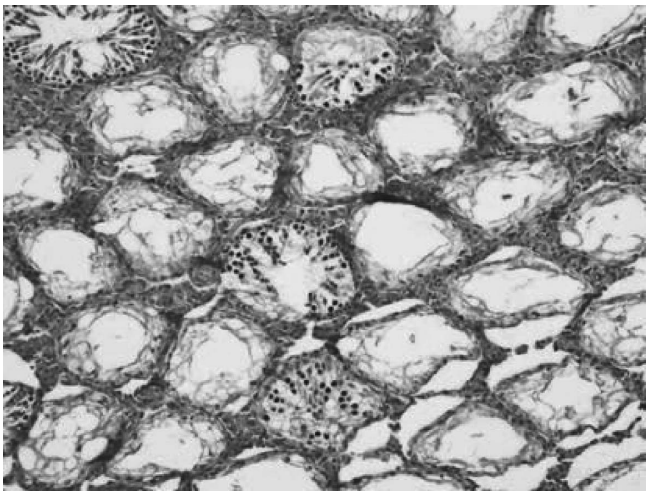

Fig. 5. Testis biopsy of F_1 rats in the high-dose group in the late stag (×100).

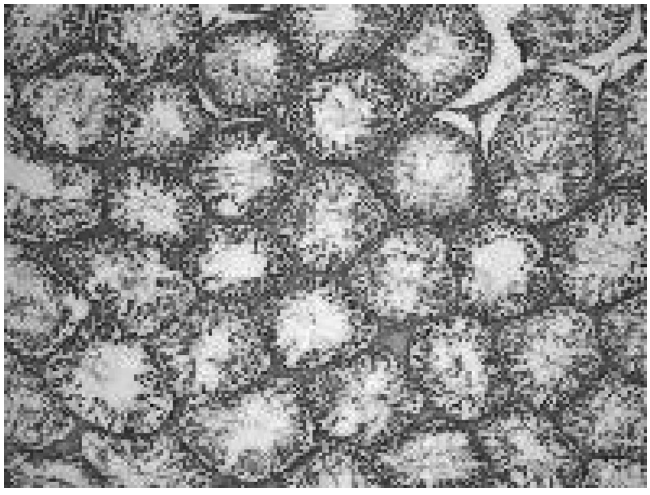

Fig. 4. Testis biopsy of F_0 rats in the low-dose group in the middle stage (×100).

cells grew well in the convoluted tubule, demonstrating a large cell volume and anachromasis thickening of the nucleolus. In the middle stage, the tube wall of the convoluted tubule increased in thickness, spermatogenic cells became reduced in number, interstitial cells were hyperplasic, and inflammatory cells infiltrated into the interstitial tissue. In the late stage, the convoluted tubules displayed an empty net structure, spermatogenic cells became reduced (in fact, very few sperm were observed), and stromal cells proliferated. With regard to the ovary, no significant anomalies were observed during the early stage. In the middle stage, some of the follicular cells became swollen and vacuolar, and then degenerated. In the late stage, few ovarian follicles were observed, and the corpus luteum was enlarged.

Changes in the high-dose group were observed mainly in the testicles/ovaries. In the early stage, the spermatogenic cell layer in the convoluted tubule was reduced, with increscent spermatogenic cell nucleoli and anachromasis thickening of the nucleolus. In addition, interstitial cells had proliferated, with infiltration of inflammatory cells into the interstitial tissue. During the middle stage, spermatogenic cells were reduced and displayed atypical changes; very few sperm were observed. In the late stage, the convoluted tubules were clearly atrophied, and vacuolisation, almost no sperm cells, and significant proliferation of the stromal cells were observed. Regarding the ovary, ovarian follicles were observed at different levels, with pycnotic nuclei, an enlarged corpus luteum, and an abundant corpus luteum during the early stage. In the middle stage, mature ovarian follicles were still present. In the late stage, ovarian follicles were atrophied, and the corpus albicans had increased.

DISCUSSION

The present study is the first to evaluate the genotoxicity induced by long-term ingestion of low-dose depleted uranium in food. We observed the uranium content in tissues, the potential damage of depleted uranium on the genetic material, and the pathomorphological changes of reproductive organs.

It is well recognized that uranium accumulates in the kidneys and bone. The results of this study also showed that after chronic oral exposure to a low dose of depleted uranium, uranium accumulated mainly in the kidney. Additionally, in the low-dose group, uranium content in F_1 rats was significantly higher than in F_0 rats, while differential accumulation was also found in the ovary. These results provide evidence that depleted uranium can be accumulated from generation to generation. To date, few studies in the literature have investigated this phenomenon.

Regarding the sperm abnormality rate, each dose of depleted uranium caused the sperm abnormality rate to increase significantly compared with the normal control group. Moreover, the sperm in the high-dose group had a malformation rate that was significantly higher than that in the low-dose group. This demonstrated that after chronic oral exposure to a low dose of depleted uranium, the sperm abnormality rate increases in rats. As the dose of depleted uranium increased, the sperm abnormality rate also increased. The results of this study also show that the sperm abnormality rate in F_1 rats was significantly higher than that in F_0 rats in the same dose group, proving that chronic oral exposure to a low dose of depleted uranium causes genetic toxicity to the sperm. Other studies[20,21] showed that the main target organs of depleted uranium in the human body are the kidney and testicles. In these studies, female rats were surgically implanted with DU tablets. During the 20 days of pregnancy, a marked increase in the depleted uranium level was observed in the female kidney, placenta, and foetus, demonstrating that toxicity can be delivered from mother to offspring. These injuries were caused by acute, large doses of depleted uranium, as described in the literature.[20,21] The present study was based on long-term intake of low doses of depleted uranium, in contrast to the previous reports based on high-dose intakes. The rate of spermatozoa abnormality will increase significantly with the amount of depleted uranium up to a certain dose. The study of genetic reproductive toxicity from enriched uranium and natural uranium revealed that dicephaly and hookless sperm could be induced by $^{235}UO_2F_2$. The amount of sperm DNA strand breakage increased with the rate of $^{235}UO_2F_2$ intake.[22] Domingo JL[21] found that histopathologic examination of the testicles of mice after 64 days of treatment did not reveal any significant differences between controls and uranium-exposed animals with regard to tubule diameter, tubule alterations, or interstitial alterations (focal atrophy, binucleated cells), with the exception of an increase in Leydig cell vacuolisation at 80 mg/kg/day. Although these changes might all have contributed to the reduction in the pregnancy rate, it is also possible that uranyl acetate treatment for 64 days produced behavioural changes (including a decrease in the libido of those animals), which in turn contributed to this reduction.

The micronucleus assay is a reliable method for evaluating the effect of genetic toxicity on chromosomes. The micronucleus rate increased after chronic oral exposure to a low dose of depleted uranium. However, there was no significant difference between the high-dose group and the low-dose group. Thus, a significant positive correlation between the micronucleus rate and the dose of depleted uranium was not detected. The micronucleus rate of the F_1 generation increased significantly more than in the F_0 generation for the same dose, demonstrating that chronic oral exposure to a low dose of depleted uranium caused genetic toxicity to the chromosomes. This result is in accord with a related report.[21,23,24] For example, Ihrulj S[23] found that one of the genotoxins present in the environment as a consequence of the last conflict in Bosnia and Herzegovina was depleted uranium, and also demonstrated increased micronuclei formation within the exposed population that showed a statistically significant correlation with age. In addition, the results of the micronucleus cytochalasin-B test for peripheral blood lymphocytes showed increased micronuclei frequencies in the exposed group.[24] The survey of staff exposure to depleted uranium showed that the carcinogenic effects of uranium enable significantly increased chromosomal abnormality, sister chromatid exchange, and micronucleus detection rates.[21] Because the peripheral blood lymphocyte micronucleus rate and the bone marrow cell micronucleus rate are widely used to determine the dose received from radiation accidents, the present study can be used for further exploration of the relationship between the dose of depleted uranium in the body over time and micronucleus rate.

The comet assay showed that the dose of depleted uranium increased along with the damage to sperm DNA, and depleted uranium resulted in genetic toxicity to sperm DNA. Other researchers[25] found that exposure to DU by inhalation resulted in DNA strand breaks in bronchoalveolar lavage (BAL) cells, and an increase in inflammatory cytokine expression and production of hydroperoxides in the lung tissue. These results suggested that the DNA damage was in part due to the inflammatory processes and oxidative stress. The effects, which seemed to be linked to the dose, were independent of the solubility of uranium compounds and correlated with the type of inhalation employed. Under neutral conditions, the comet assay revealed that DNA damage in BAL cells was composed partly of double strands breaks, suggesting that radiation could contribute to DU genotoxic effects in vivo. This assay can detect cellular damage and repair below the 2 Gy irradiation threshold, and it is considered as the most rapid and sensitive detection method for low-dose radiation exposure (0.05 Gy).[26]

We have also shown in the present study that Wistar rats are a good model for long-term feeding with different doses of depleted uranium in order to observe different pathological changes in parents and offspring. Our results show that disease was mainly concentrated in the reproductive organs. After chronic oral exposure to a specific dose of depleted uranium, pathological changes were observed in the testicles, and with increasing doses and time of intake, the damage became more obvious. When a certain dose was reached, spermatogenic cells displayed atypical changes, and the testicles were more sensitive than the ovaries to injury in response to depleted uranium.

In short, after chronic oral exposure to a low dose of depleted uranium, the genetic material of F_0 and F_1 rats changed. Further studies could explore the relationship

among the changes in the genetic material, the dose of depleted uranium in the body, and uranium intake over time. The establishment of a dose-response relationship equation would provide a basis for further diagnosis and the prevention of injury caused by depleted uranium.

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