Vitamin Status and Spermatogenesis in Rats during Late Stages after Irradiation in Various Doses

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The content of vitamin B_2 in testes increased 6.5 months postirradiation irrespective of the irradiation dose. The concentration of vitamin E decreased. The least pronounced decrease was observed in animals irradiated with 0.5 Gy; these changes persisted for 15.5 months. The concentration of spermatozoa, the weight of epididymides and body weight increased. No changes in angiotensin-converting enzyme activity were found. Histological changes in the testes attest to inhibited growth of the spermatogenic epithelium in convoluted seminiferous tubules, which depended on the irradiation dose.

Key Words: *irradiation; rats; vitamins; spermatogenesis*

Over the last decade, andrologic consequences of the Chernobyl' accident have received little attention. However, there are data on the effects of irradiation on not only liquidators, but also their progeny. Irradiation affects fertility and survival rates in exposed animals and the development and reproductive potency of offspring. Long-term irradiation effects and reactions of some systems and organs are well known [6,7]. However, the intensity of spermatogenesis in various postirradiation periods, the dependence of reproductive system functions on the irradiation dose, and the involvement of antioxidants in reproductive system reactions to irradiation remain unclear.

The dynamics of disturbances of the antioxidants and reproductive system and after irradiation in a dose of 50 cGy was demonstrated [3]. In the present study, the animals were exposed to irradiation in various doses, and the reproductive system was examined in late postirradiation periods.

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MATERIALS AND METHODS

Male adult Wistar rats weighing 250-350 g et the age of months were kept under standard vivarium conditions. The animals were divided into 5 groups and exposed to a single irradiation in doses of 0.25 Gy (group 1), 0.5 Gy (group 2), and 1 Gy (group 3). Tissue samples were taken 15.5 months postirradiation. In group 4 rats exposed to 1-Gy irradiation, biopsy was performed 6.5 months postirradiation. Group 5 animals were controls (biopsy was performed after 6.5 months).

Irradiation was conducted on a Luch device (^{60}Co, Medical Radiology Research Center).

After intraperitoneal administration of 0.25 ml 5% sodium thiopental, the animals were weighed, and the testes and epididymides were isolated. Epididymides were weighed and minced in 20 ml physiological saline. The suspension was centrifuged in a cold centrifuge at 2000 rpm for 25 min to obtain spermatozoa. The supernatant was removed and the precipitate was resuspended in 1 ml buffer (pH 7.4) and stored at -8°C before angiotensin-converting enzyme (ACE) assay [2]. The blood (5 ml) was taken from the inferior vena cava and centrifuged at 1500 rmp for 10 min. The

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serum was stored at -8°C. The epididymis and one liver sample were stored at -40°C.

The content of vitamin B_2 was determined by titration of serum, liver, and testicular riboflavin with riboflavin-binding apoprotein [5]. The content of vitamin B_6 was determined by the concentrations of pyridoxal coenzymes (pyridoxal phosphate and pyridoxal) in the serum measured by high-performance liquid chromatography [10]. Vitamins A and E in the serum and epididymis were analyzed by high-performance liquid chromatography [9].

The concentration of spermatozoa was determined as described previously [4]. Convoluted seminiferous tubules (CST) containing (per 100 CST cross-sections) 1 layer (spermatogonia), 2 layers (spermatogonia and spermatocytes), and 3-4 layers (spermatogonia, spermatocytes, spermatids, and spermatozoa) were counted on histological sections of testes. The number of CST containing no gametes (empty CST) or including desquamated epithelium was also estimated. The percentage of each type of CST was determined. The index of spermatogenesis (total number of layers divided by 100) was calculated [8,12].

RESULTS

Liver content of vitamin B_2 increased 15.5 months postirradiation and was maximum (a 1.8-fold increase) in animals irradiated in a dose of 0.5 Gy. Testicular level of vitamin B_2 increased by 40% after irradiation in a dose of 0.25 Gy. Similar effect was observed after irradiation in other doses. Serum concentration of vitamin B_2 did not differ from the control.

The contents of pyridoxal coenzymes and retinol in the serum did not depend on the irradiation dose and remained unchanged.

Serum tocopherol concentration was constant irrespective of the irradiation dose. However, the content of tocopherols in the epididymis decreased after irradiation and reached the minimum at a dose of 0.5 Gy. This probably resulted from direct interaction between vitamin E and free radicals intensively generated after irradiation. Vitamin E plays the major role in the protection of spermatozoon membrane from lipid peroxidation [13] impairing its fertilization potencies [11].

The decrease in the content of testicular tocopherols and increase in vitamin B_2 concentrations in the testis and liver appeared on the 7th month and persisted to the 16th month postirradiation indicating long-term disturbances in the vitamin status leading to latent diseases. Similar changes were observed in liquidators of Chernobyl' accident 6-8 years postirradiation (neurocirculatory asthenia, decreased libido, diminished erection, pathozoospermia, and chronic congestive prostatitis) [3].

Apart from these changes in vitamin status, the concentration of spermatozoa, the weight of the epididymides, and body weight increased. An increase in the weight of epididymides was the most pronounced after irradiation in doses of 0.25 and 0.5 Gy, while the body weight was maximum after irradiation in a dose of 0.5 Gy. Our findings indicate that these doses produce a minor stimulating effect on muscle growth in rats.

ACE activity in spermatozoa of irradiated rats did not differ from the control levels, did not depend on the irradiation dose and the period of examination, and varied from 22 to 27 U/million cells (the mean level was 25±2 U/million cells). ACE activity in the serum varied in a broader range, did not depend on the irradiation dose, and was 1.5-2 times lower than in spermatozoa.

Such stability of ACE may result from its radiational resistance in this dose range. Probably, our ex-

TABLE 1. Spermatogenesis in Irradiated Rats (Number of CST with Gametes at Various Stages of Maturation per 100 CST, *M±m*)

Conditions	3-4 layers	2 layers	1 layer	Empty CST	Desquamation of gametes into CST	Index of spermatogenesis
Control (n=4)	97.3±0.35	1.3±0.24	0.6±0.12	0.8±0.14	3.5±0.31	3.89±0.14
6.5 months postirradiation (1 Gy, *n*=4)	91.3±1.3*	2.5±0.86	2.5±0.86	3.0±1.4	26.0±1.23*	3.74±0.05*
15.5 months postirradiation (1 Gy, *n*=4)	95.0±1.17	2.3±0.55	1.0±0.58	1.6±0.34*	18.6±1.89*	3.85±0.03
15.5 months postirradiation (0.5 Gy, *n*=3)	60.6±24.07	12.9±6.78	10.4±5.63	16.1±8.62	19.3±4.55*	2.27±0.98
15.5 months postirradiation (0.25 Gy, *n*=3)	98.6±0.33*	0.33±0.19	0*	1.0±0.58	17.0±3.74*	3.95±0.02*

Note. *$p<0.05$ compared with the control.

periments were not absolutely appropriate for studying ACE. Studies performed on lower mammals and primates are known to give different results. Thus, extrapolation of results obtained in experiments on primates to humans is more adequate [1].

The histological analysis of the testes revealed changes induced by irradiation (Table 1). Irradiation with 0.5 Gy and 1 Gy changed the index of spermatogenesis (compared with the control). Some empty CST (without spermatogenic epithelium or containing desquamated cells) were often seen. Irradiation in a dose of 0.25 Gy produced less pronounced changes. Thus, irradiation of rats during differentiation of spermatids to spermatozoa induced desquamation of gametes into the CST lumen.

The decrease in testicular vitamin E content indicates that specific conditions promoting to free radical generation are maintained in testes for a long post-irradiation period. This is indirectly confirmed by the fact that in liquidators of consequences of the Chernobyl' accident, the number of pathologically changed spermatozoa remained increased during 6-8 years post-irradiation. In the majority of cases (40%), the spermatozoon head was damaged. Simultaneously, the number of immature gametes in the semen increased to 6-8% (normally 1-2%).

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