Effect of Irradiation on Vitamin Status and Spermatogenesis in Rats

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 123, No. 5, pp. 524-527, May, 1997
Original article submitted February 22, 1996

It is shown that in rats exposed to single external irradiation in a dose of 0.5 Gy the concentration of spermatozoa surpasses that of nonirradiated controls. The level of vitamin B_2 increases in the liver and testes of irradiated animals. For the given dose of ionizing radiation, the participation of angiotensin-converting enzyme in cell maturation during spermatogenesis is not observed. Various changes in the content of antioxidant vitamins at different stages of the experiment are described.

Key Words: *spermatozoa; irradiation; angiotensin-converting enzyme; vitamins; antioxidants*

Radiation produces a variety of biological effects on mammals and men. Numerous studies demonstrated a dose-dependent effect of external irradiation on all systems of the organism. However, little is known on the mechanisms of this effect. Organism's response to irradiation include the repair processes. The efficiency of these processes has been little studied [8].

The reproductive system, gonads, and cells of spermatogenesis are very sensitive to radiation. We previously described [3,4] the effect of irradiation on the reproductive system in Chernobyl cleanup workers. Analysis of spermograms revealed a rise of morphologically abnormal spermatozoa and the presence of numerous immature cells in ejaculate. At the same time, we found elevated activity of angiotensin converting enzyme (ACE) in spermatozoa, however, this phenomenon has not been explained. Taking into account that the Chernobyl workers were examined on average 4 years after irradiation (the mean dose in the group was 0.158 Gy), we decided to undertake an experimental study for evaluation of immediate effects of various doses of radiation.

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In the present study we explore changes in the intensity of spermatogenesis, ACE activity in spermatozoa, and the content of vitamins A, E, B_2, and B_6 in the testes, serum, and liver of mature rats exposed to external irradiation in a dose of 0.5 Gy.

MATERIALS AND METHODS

Irradiation was performed using an IGUR apparatus (Institute of Biophysics, Russian Academy of Medical Sciences): ^{137}Co emitter, 2.53 rad/sec power, 0.5 Gy (50 rad) dose, and 18 sec exposure. Age-matched animals served as the control.

ACE activity was measured as described elsewhere [3]. Spermatozoa were counted as follows: epididymis homogenate in 10 ml physiological solution was centrifuged at 1500 rpm for 20 min, and the resultant supernatant was centrifuged at 2000 rpm for 30 min. The sediment was resuspended in 1 ml buffer (pH 7.4), and the cells were counted in a Goryaev chamber (×200). In parallel, cells suspended in buffer containing a detergent phenylmethylsulfonyl fluoride were counted. It was shown that detergent did not increase ACE activity.

For characterization of vitamin metabolism the following parameters were determined. Vitamin B_2: riboflavin content in the testes and liver was mea-

0007-4888/97/0005-0456$18.00

sured by titration with riboflavin-binding apoprotein [5]. Vitamin B_6: serum contents of specific coenzymes (pyridoxal phosphate, PP, and pyridoxal, P) were measured by high-performance liquid chromatography [9]. Vitamins A and E were measured by the same method [10].

RESULTS

It has been shown that irradiation in a dose of 50 rad does not cause sterilization; however, the effects of such an irradiation are less studied than those of higher doses [7]. Tissue specimens were obtained at different times after irradiation on the basis of published data [13].

All studied parameters rose during a 3-month observation period (Table 1). The level of spermatogenesis increased and considerably surpassed the initial and final control values. Body weight increased. The mass of epididymis rose 1.5-fold by the end of observation, while the concentration of spermatozoa surpassed 2-fold the initial value. At stage I of the experiment (1 week postirradiation), the parameters of spermatogenesis in irradiated animals did not differ from the initial values in the control, while at stage IV they were higher than the final values in the control rats.

ACE activity in spermatozoa from rats exposed to low dose irradiation remained unchanged throughout the observation period and did not differ from that in nonirradiated rats: 25±8 U/10^6 cells. It should be noted that in rat spermatozoa ACE activity was considerably higher than in human spermatozoa (3.1±1.5 U/10^6 cells) [3].

The elevated ACE activity in Chernobyl cleanup workers was noted at an average dose of 0.158 Gy, which dictates a necessity of varying the ionizing radiation dose in further studies on experimental animals.

Ionizing radiation induces generation of free radicals potentiating its damaging effect. Recent investigations showed that peroxidation plays a key role in etiology of male sterility [11,12]. In particular, an inverse correlation was found between the intensity of reactive oxygen radicals and functional state of human sperm. Many vitamins (E, C, and carotenoids), and endogenous natural nonenzymatic components of the antioxidant system exert a protective effect against ionizing radiation. This is confirmed by the fact that vitamin deficiency is accompanied by elevated sensitivity to radiation [1,6]. *In vitro* experiments have demonstrated a protective effect of vitamin E and ascorbic acid against lipid peroxidation [14]. It was previously reported that irradiation affects organism's saturation with other vitamins; however, published data are contradictory due to the use of different doses of ionizing radiation and different times after irradiation.

The contents of water- and lipid-soluble vitamins in some rat organs are presented in Table 2. Serum content of retinol after irradiation decreased and attained the minimum (53% of the control value at the start of the experiment) by days 16-22 (stage II). This parameter slightly rose (to 64% of the initial level) by days 36-42 postirradiation (stage III) and on day 90 postirradiation (stage IV) it did not differ from the final values in the control animals. It should be noted that in intact animals the content of vitamin A in the serum and testes decreased with age ($p<0.1$ and $p\leq0.05$, respectively). In the testes of irradiated animals retinol content decreased by 42% in comparison with the control animals and remained at this level throughout the observation period.

The total serum content of tocopherols dropped 3.5-fold on days 16-22 postirradiation in comparison with the control. On day 40, this parameter increased to 75% of the control value, while on day 90 it did not differ from the control. It should be noted that in intact rats the vitamin E content increased by 40% with age. In irradiated animals, no considerable changes in the total tocopherol content were noted.

The content of vitamin B_2 in the liver in both groups was about 20 μg/g wet tissue, which corresponded to consumption of 20 μg riboflavin/rat per day [8]; a full-value semisynthetic ration should contain 100 μg. Thus, the animals maintained on the vivarium ration have an initial vitamin B_2 deficiency.

TABLE 1. Spermatogenesis, Body Weight, and Weight of Epididymes at Different Times Postirradiation ($M\pm m$, n=9-12)

Stage	Experiment			Control		
	body weight, g	weight of epididymis, mg	concentration of spermatozoa, 10^6/ml	body weight, g	weight of epididymis, mg	concentration of spermatozoa, 10^6/ml
I	199±42	744±228	14.8±3.8	210±34	763±82	—
II	231±37	724±220	21.4±5.1	—	—	—
III	332±35	1087±83	24.5±2.4	—	—	—
IV	336±29	1183±77	29.4±2.2	368±22	1273±194	23.3±1.9

TABLE 2. Content of Vitamins A, E, B_2, and B_6 in the Blood, and Testes at Different Times Postirradiation ($M\pm m$, n=6-14)

Parameter	Experiment				Control	
	I	II	III	IV	I	IV
Vitamin A						
Serum, μg/dl	20.5±4.3	12.8±2.7	15.5±2.7	18.2±2.4	24.2±1.9	19.7±1.5
Testes, μg/g	0.20±0.05**	0.27±0.05	0.24±0.03	0.19±0.04	0.35±0.03	0.26±0.02*
Vitamin E						
Serum, μg/dl	0.20±0.02	0.07±0.02**	0.18±0.03	0.35±0.04*	0.24±0.03	0.33±0.03*
Testes, μg/g	8.5±0.9	10.5±2.6	9.1±0.8	9.5±0.9	10.3±1.6	10.2±0.8
Vitamin B_2						
Testes, μg/g	3.28±0.16	3.78±0.26	3.70±0.14	4.17±0.16***	3.17±0.14	2.84±0.12
Vitamin B_6						
Serum, μg/dl	118.1±8.9**	152.9±23.6	142.0±15.2	134.7±18.3**	163.5±13.0	206.5±8.4*

Note. $p\leq0.05$: *compared with the age in control group; **compared with control group; ***compared with the age in experimental group.

On days 16-22 postirradiation, we observed a tendency towards an increase in the total riboflavin content in the testes and on day 90 this parameter rose by 27%. A more pronounced rise in the content of riboflavin was found in the liver: on day 90 it increased 2-fold. This effect was not age-related but most likely caused by irradiation, since in intact animals the content of vitamin B_2 in the studied organs remained practically unchanged throughout the observation period.

Seven days postirradiation, the total content of pyridoxal coenzymes in rat serum decreased by 28% in comparison with the control (Table 2). Starting from day 16, this parameter slightly rose and then remained at this level during the entire experiment. In intact animals the total serum content of PP and P considerably rose and therefore this parameter in irradiated animals was far below the control values.

Irradiation in the chosen dose did not disturb spermatogenesis in random-bred rats. Moreover, by the end of the experiment the concentration of spermatozoa in irradiated animals surpassed that in the control group. In light of this, the applied dose (0.5 Gy) can be regarded as a spermatogenesis-stimulating dose. Contrary to our expectations, ACE was not actively involved in cell maturation during spermatogenesis, this effect was not found. At the same time, ACE activity in rat was 8-fold higher than in man, which attests to species-specific differences of this enzyme.

The content of vitamin E in the testes was more than one order of magnitude higher than the concentration of vitamin A. The contents of vitamins A and E remained practically unchanged during the observation period, while in the serum we found a decrease in these parameters followed by restoration of the normal. Vitamin E occurring in the cell membrane interacts with fatty acid radicals. Spermatozoa are rich in fatty acids. Taking into account a high content of abnormal spermatozoa (especially spermatozoa with abnormal head) in the spermograms of Chernobyl workers, it can be hypothesized that the interaction between the antioxidant and radicals occur within the cell membrane.

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