Modification of Radiation-Induced Changes in the Reproductive System of Male Rats with Sodium Succinate

G. G. Vereshchako, A. M. Khodosovskaya, O. V. Artemenko, and E. F. Konoplya

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We studied the effects of sodium succinate on the reproductive system and blood leukocyte count in rats 30 and 60 days after fractionated irradiation in a dose of 1 Gy. Sodium succinate in concentrations of 0.2 and 0.5% was given *ad libitum* with water before (1 week) and during irradiation (5 days). The preparation promoted morphofunctional recovery of the testes and epididymides and restored the count of peripheral blood leukocytes.

Key Words: *fractionated irradiation; sodium succinate; weights of testes and epididymides; spermatogenic cells; DNA; RNA; dehydrogenases; blood leukocytes*

The reproductive system is highly sensitive to ionizing radiation [3,10]. Long-lasting functional disturbances in the pituitary-gonadal system were found in liquidators of the Chernobyl accident (absorbed dose 0.05-1 Gy) [6]. The search for new substances protecting the reproductive system during irradiation and producing no side effects is of considerable importance. Some oxidation substrates hold much promise in this respect [12]. Previous studies showed that succinic acid (succinate) produces a protective effect during lethal irradiation [2,4,14]. The efficiency of succinate in low-dose irradiation is poorly studied. Moreover, the effects of succinate on the reproductive system during irradiation were not studied.

Here we studied the modifying effect of sodium succinate on the testes and epididymides in rats exposed to fractionated irradiation in a dose of 1 Gy. General state of animas was estimated by the peripheral blood leukocyte count.

MATERIALS AND METHODS

Experiments were performed on male albino rats aging 1 month and weighing 63.7±7.4 g. Some animals were daily exposed to fractionated γ-irradiation in a dose of 0.2 Gy on an IGUR-1 device (^{137}Cs, 5.0 cGy/min, total dose 1 Gy). Group 1 rats received no correction therapy. Group 2 and 3 rats received sodium succinate in concentrations of 0.2 and 0.5% (*ad libitum* with water), respectively, before (1 week) and during irradiation. Intact rats served as the control. On days 30 and 60 after irradiation, 6 rats from each group were weighted and decapitated. The blood was collected. Leukocytes were counted routinely. The testes and epididymides were isolated and weighted. The tunica and blood vessels were removed from one testis and cell suspension was prepared [5]. Spermatozoa were isolated from the epididymis [1]. The total count of sex cells and number of spermatozoa in the testis and epididymis were counted in a Goryaev chamber. Another testis was used to prepare 10% homogenate. Mitochondria were isolated by centrifugation in 0.25 M sucrose and Tris buffer (pH 7.4) at 12,000*g* for 15 min [7]. Activity of succinate dehydrogenase (SDH, EC 1.3.99.1) was measured [11]. Total activity of lactate dehydrogenase (LDH, EC 1.1. 1.27) was measured in the cytoplasmic fraction of testes and spermatozoa isolated from the epididymides. Protein content was measured by the method of Lowry [13]. The content of RNA and DNA was determined in testis homogenate (10%) [8,9].

The results were analyzed by Student's *t* test.

Institute of Radiobiology, Belarussian Academy of Sciences, Minsk

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RESULTS

Fractionated irradiation in a dose of 1 Gy significantly decreased the weights of reproductive organs (Table 1). The relative weights of the testes and epididymides decreased to 67.0 and 82.7% of the control, respectively, on day 30 after irradiation. Then the weights of these organs progressively decreased. Sodium succinate normalized the relative weights of testes and, to a lesser extent, of the epididymides. On day 60 the relative weight of the testes in rats of groups 2 and 3 reached 125.4 and 133.3%, respectively (compared to group 1 animals).

On day 30 after irradiation, blood leukocyte count practically did not differ from the control, but on day 60 moderate leukopenia was observed (Table 1). Sodium succinate increased leukocyte count on day 30 after irradiation and normalized this parameter by day 60.

The total count of spermatogenic cells and spermatozoa in irradiated and control rats on days 30 and 60 after irradiation differed insignificantly. In group 1 and 2 rats the count of spermatozoa in the testicular tissue tended to increase on day 30. This effect was not observed 60 days after irradiation.

The count of spermatozoa slightly increased 30 days after fractionated low-dose irradiation. However, spermatogenesis was sharply suppressed on day 60. At this term the count of spermatozoa in the epididymides from group 1 rats was 39.3% of the control. Sodium succinate had no effect on the count of spermatozoa at any stage of observations.

RNA content in the testicular tissue changed insignificantly on days 30 and 60 after irradiation. In these periods DNA content increased by 16.6 and 18.9%, respectively. LDH activity in the testicular tissue remained practically unchanged in group 1 rats. How-

TABLE 1. Effects of Sodium Succinate on Blood Leukocyte Count and Morphofunctional State of the Testes and Epididymides in Rats Exposed to Fractionated Irradiation in a Dose of 1 Gy ($M \pm m$, n=6)

Parameter; period of observations, days		Intact	Irradiation with 1 Gy		
			no correction	+sodium succinate, %	
				0.2	0.5
Peripheral blood leukocytes,	30	4.83±0.65	4.60±0.65	5.40±0.65	6.64±0.81
10^9/liter	60	6.08±0.85	5.12±0.39	6.04±0.37	6.00±0.62
Testes					
Relative weight, %	30	0.701±0.050	0.470±0.020*	0.521±0.030*	0.453±0.040*
	60	0.670±0.060	0.417±0.020*	0.523±0.040	0.553±0.010
Spermatozoon count, 10^8/g	30	1.27±0.08	1.42±0.05	1.52±0.10	1.65±0.08*+
	60	1.73±0.23	1.32±0.05	1.37±0.09	1.32±0.15
Spermatogenic cell	30	3.22±0.10	3.11±0.08	3.29±0.13	3.24±0.07
count, 10^8/g	60	3.78±0.44	3.70±0.11	3.58±0.15	3.82±0.06
RNA, mg/g	30	1.93±0.06	1.82±0.08	1.80±0.10	1.88±0.08
	60	2.10±0.06	2.17±0.08	1.86±0.04	2.06±0.09
DNA, mg/g	30	2.59±0.22	3.02±0.32	2.78±0.15	2.75±0.03
	60	3.09±0.12	3.67±0.18	4.42±0.21	4.05±0.13
LDH, μmol pyruvate/mg	30	0.036±0.001	0.036±0.002	0.034±0.002	0.035±0.001
protein/min	60	0.036±0.001	0.039±0.002	0.033±0.002+	0.032±0.001
SDH, μg/mg protein/min	30	4.55±0.20	4.14±0.34	3.63±0.38	5.42±0.43+
	60	5.65±0.19	7.44±0.46*	5.65±0.21+	6.35±0.31
Epididymis					
Relative weight, %	30	0.161±0.004	0.129±0.003*	0.143±0.002*+	0.129±0.007*
	60	0.148±0.004	0.109±0.001*	0.124±0.006*+	0.121±0.001*
Spermatozoon count, 10^6/g	30	9.40±0.29	10.80±0.14*	10.90±0.18*	9.55±0.39+
	60	17.9±0.38	7.04±0.25*	6.05±0.27*+	7.77±0.55*
LDH, μmol/mg protein/min	30	0.043±0.002	0.033±0.002*	0.040±0.001+	0.038±0.002
	60	0.042±0.001	0.034±0.002*	0.036±0.003*	0.032±0.001

Note. p<0.05: *compared to intact rats; +compared to irradiation without correction.

ever, SDH activity considerably increased 60 days after irradiation (131.7%). LDH activity in spermatozoa isolated from the epididymides was low on days 30 and 60 after irradiation (Table 1).

In rats receiving sodium succinate, the content of DNA in testes, LDH activity in spermatozoa, and SDH activity in testicular mitochondria (group 3 animals) returned to normal on day 30. Moreover, sodium succinate normalized SDH activity and increased DNA content in the testicular tissue on day 60 after irradiation. This was probably related to an increase in the ratio of diploid sex cells and count of interstitial cells.

Our results indicate that sodium succinate in concetrations of 0.2 and 0.5% improves resistance of the reproductive system to low-dose irradiation. Sodium succinate acts as the oxidation substrate during irradiation-induced hypoxia and modulates lipid peroxidation in mitochondria [14].

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