The Use of Micronucleus Test For Detection of Genotoxic Damage to the Thyroid Gland

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We studied the possibility of using the micronucleus test in *in vivo* experiments on the model of rat follicular thyrocytes prestimulated to cell division (hemithyroidectomy). Single administration of N-nitroso-N-methylurea produced a significant dose-dependent effect on micronucleus formation in thyrocytes and polychromatophilic erythrocytes of the bone marrow. The test system allowed us to reveal a cumulative effect of 2-fold and 4-fold treatment with the mitogen in low or subthreshold doses on the thyroid gland. Our results indicate that the micronucleus test is an informative method for the analysis of the effect of genotoxic agents on the thyroid parenchyma.

Key Words: *thyroid gland; thyrocytes; micronucleus test; nitroso methylurea*

The micronucleus test is an express method of detection of genotoxic damage to the thyroid gland. This test is based on calculation of interphase cells with additional nucleoli (micronuclei). Micronuclei are formed from isolated fragments or whole chromosomes under the influence of genotoxic clastogens and aneugens during prior mitosis [2,6]. Continuously dividing populations are extensively used for this purpose. Counting of micronuclei in bone marrow erythroid cells is a standard express test for genotoxicity of chemical compounds [6]. Highly specialized populations pretreated for stimulation of cell division serve as a test system for detection of genetic abnormalities formed over a long period of time. In this respect much attention was given to hepatocytes [3,11,12]. The follicular epithelium of the thyroid gland (TG) is another promising test system. Various technogenic factors (mutagens, radionuclides, *etc*.) can induce pathological changes in TG, which is accompanied by stimulation of division and damage to the genome in glandular cells [4,10]. Micronucleus formation in cultured thyrocytes under the influence of radiation illustrates the necessity to perform detailed investigations of this problem [8,9].

Here we evaluate informativeness of the micronucleus test as a biological indicator of chemical mutagenesis in the thyroid epithelium induced with N-nitroso-N-methylurea *in vivo*.

MATERIALS AND METHODS

Experiments were performed on 60 adult male Wistar rats weighing 200-250 g. Left-sided hemithyroidectomy was used for stimulation of thyrocyte division [1]. The surgery was performed on rats narcotized with hexenal. Surgically removed parts of TG from 10 rats served as the control. We performed 2 experimental series. In series I the animals received single intraperitoneal injection of N-nitroso-N-methylurea (Sigma) in doubling doses of 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, and 12.8 mg/kg 48 h after surgery. Each group consisted of 4-7 rats. This compound has high clastogenic activity. Under experimental conditions N-nitroso-N-methylurea induces tumor growth in TG [7]. Cumulative effect

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was studied in series II. Some operated animals received low doses of the mutagen (0.1 and 0.2 mg/kg) on days 2 and 4, while others were treated on days 2, 3, 4, and 5. The rats were killed with ether vapors on days 4 (series I) and 9 after surgery (series II). Thyrocytes were isolated by dissociation of TG fragments in 0.25% collagenase for pancreatic islet isolation (Sigma) at 37°C for 2-2.5 h. Smears of cells from TG and bone marrow were fixed with ethanol. Cell smears were stained by the method of Felgen or treated with gallocyanine-chrome alum (method of Einarson). The cytoplasm was stained with light green or eosin. We estimated the ratio of micronucleated thyrocytes and polychromatophilic erythrocytes (PCE) per 1000-2000 cells from each organ. The mean number of micronuclei per micronucleated cell was calculated.

RESULTS

The structure of micronuclei in thyrocytes varied. The structure and density of chromatin were similar in relatively large micronuclei and main nuclei (Fig. 1, *a*). Relatively small micronuclei were mainly composed of fine euchromatin and looked like more light structures (Fig. 1, *b*). We showed that 94% micronucleated cells have 1 micronucleus, and not more than 6% micronucleated cells enclose 2 micronuclei.

The subthreshold dose of the mutagen for both populations was 0.1 mg/kg (Table 1). The mutagen in doses of 0.2-3.2 and 0.2-0.8 mg/kg produced a significant dose-dependent effect on micronucleus formation in thyrocytes and PCE, respectively. The process of micronucleus formation in thyrocytes and PCE became stable after administration of the

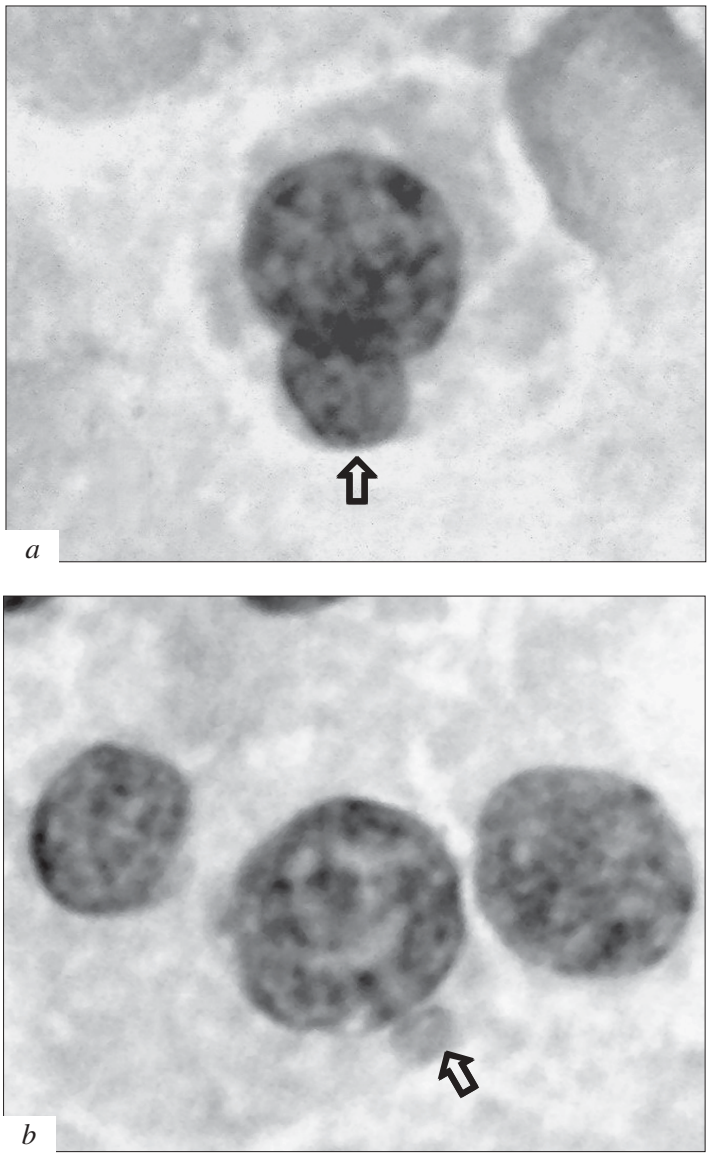

Fig. 1. Isolated micronucleated thyrocytes (arrows). Felgen and light green staining, ×630.

TABLE 1. Micronucleus Formation in Thyrocytes and Red Bone Marrow PCE after Single Administration of Mutagen ($X \pm S_x$)

Mutagen dose, mg/kg	Thyrocytes			
	micronucleated cells, %	mean number of micronuclei per micronucleated cell PCE	micronucleated cells, %	mean number of micronuclei per micronucleated cell
Control	0.19±0.03	1.00	0.31±0.08	1.08
0.1	0.29±0.04	1.00	0.47±0.13	1.12
0.2	0.30±0.03*	1.00	0.83±0.08*+	1.00
0.4	0.57±0.06*+	1.04	0.86±0.14*	1.08
0.8	0.92±0.07*+	1.12	1.24±0.10*+	1.07
1.6	1.36±0.12*+	1.11	1.15±0.15*	1.14
3.2	2.19±0.20*+	1.13	1.13±0.12*	1.07
6.4	2.17±0.18*	1.19	1.18±0.12*	1.03
12.8	2.34±0.13*	1.32	1.37±0.20*	1.13

Note. $p<0.05$: *compared to the control; +compared to a lower dose.

TABLE 2. Micronucleus Formation in Thyrocytes after Repeated Treatment with the Mutagen in Low Doses ($X \pm S_x$)

Total dose, mg/kg	Single dose, mg/kg	Number of injections	Micronucleated cells, %	Mean number of micronuclei per micronucleated cell
0.4	0.4	1	0.57±0.06	1.04
	0.2	2	0.56±0.05	1.11
	0.1	4	0.64±0.05	1.24
0.8	0.8	1	0.92±0.07	1.12
	0.4	2	1.11±0.11	1.08
	0.2	4	0.94±0.07	1.18

mutagen in doses of 6.4 and 1.6 mg/kg, respectively. Treatment with the mutagen in high doses did not produce further increase in the number of micronucleated cells. It was probably associated with an increase in the frequency of lethal mutations and elimination of cells from the population. Administration of N-nitroso-N-methylurea in higher doses was accompanied by an increase in the mean number of micronuclei per TG micronucleated cell. These changes reflect an increase in the frequency of chromosomal aberrations.

Injection of the mutagen in low doses affected micronucleus formation in thyrocytes from hemithyroidectomized rats (similarly to single injection of N-nitroso-N-methylurea in the same total dose, Table 2). In animals receiving one total dose of N-nitroso-N-methylurea the count of micronuclei per micronucleated cell increased proportionally to the number of injections.

Our results show that the micronucleus test is equally informative to follicular thyrocytes pretreated to stimulate cell division and erythroid cells of the bone marrow. The developed test system allows studying the effect of single treatment with mutagens in a wide range of doses. Moreover, this system can be used to reveal a cumulative effect of the mutagen in low or subthreshold doses on TG. The micronucleus test is an informative method for evaluation of the effect of genotoxic agents on the thyroid parenchyma.

REFERENCES

1. A. N. Gansburgskii, T. L. Miro, E. N. Anashkina, and M. A. Gansburgskii, *Morfologiya*, **117**, No. 3, 35 (2000).
2. A. D. Durnev and S. B. Seredenin, *Mutagens (Screening and Pharmacological Prevention)* [in Russian], Moscow (1998).
3. I. V. Uryvaeva and G. V. Delone, *Ontogenez*, **23**, No. 4, 370-377 (1992).
4. *Thyroid Gland. Fundamental Aspects* [in Russian], Eds. A. I. Kubarko and S. Yamashita, Minsk, Nagasaki (1998).
5. J. Ashby, M. D. Waters, J. Preston, *et al*., *Mutat. Res*., **352**, Nos. 1-2, 153-157 (1996).
6. M. Fenech, *Ibid*., **455**, No. 1-2, 81-95 (2000).
7. N. Fujimoto, Y. Sakai, and A. Ito, *Carcinogenesis*, **13**, No. 8, 1315-1318 (1992).

8. L. M. Green, B. M. Bianski, D. K. Murray, *et al*., *Radiat. Res*., **163**, No. 2, 172-182 (2005).
9. L. M. Green, D. K. Murray, A. M. Bant, *et al*., *Ibid*., **155**, No. 1, 32-42 (2001).
10. G. Tallini, *Endocr. Pathol*., **13**, No. 4, 271-288 (2002).
11. A. D. Tates, M. L. van de Poll, M. van Welie, and S. J. Ploem, *Chromosomal Aberrations*, Eds. G. Obe and A. T. Natarajan, Berlin (1990), pp. 242-259.
12. I. V. Uryvaeva and G. V. Delone, *Mutat. Res*., **334**, No. 1, 71-80 (1995).