Mutagenic Activity of Acrylamide in the Rat Thyroid Cells under Conditions of a Subacute Experiment

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Experimental mutagenic effect of acrylamide on thyroid gland cells was studied by an extended micronucleus test. Acrylamide in doses corresponding to 0.004-0.1 LD_{50} increased the incidence of thyroid follicular cells (A-cells) with micronuclei and other karyological parameters in exposed rats after hemithyroidectomy. This cytogenetic effect allows regarding acrylamide as a mutagen for the thyroid gland and as a carcinogen for this organ.

Key Words: *thyroid gland; acrylamide; cytogenetic disorders; binuclear cells; micronuclei*

Acrylamide (AA), highly prevalent in the environment, is a carcinogen for thyroid gland (TG). Its carcinogenic effect is assumed to be due to its genotoxicity. However, there are in fact no methods for evaluation of mutagenic activity on TG cells *in vivo* and hence, mutagenic activity of AA has never been studied on TG cells of laboratory animals. In few studies, the problem was studied using the comet assay [9], recording just the pre-mutation injuries to the genome, specifically, DNA aberrations. The micronucleus test was proposed for evaluation of the mutagenic effect [4]. Some authors recommend an expensive enzyme, collagenase, for dissociation of TG cells [4,5]. We have used polyorgan micronucleus test with cell fixation with formalin and alkaline dissociation. In addition to the analysis of micronuclei, we evaluated a wide spectrum of other karyological parameters, this appreciably improving the informative value of the method [3,6,7]. We used this approach for the first time for the analysis of TG cells [1].

Here we evaluated mutagenic activity of AA in TG, a target organ for its carcinogenic effect.

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MATERIALS AND METHODS

The study was carried out on male Wistar rats (170-240 g) in accordance with the regulations of the European Convention for Protection of Vertebrates Used for Experimental and Other Purposes (Strasbourg, 1986) and Regulations for Studies on Experimental Animals (Supplement to the Order of the Ministry of Health of the USSR, August 12, 1977). The animals were kept on standard diets with free access to water and food.

TG is an organ with slowly regenerating cell populations [4,5]. Therefore, in order to detect micronuclei, proliferative activity of TG had to be stimulated by hemithyroidectomy (HTE). Left-side HTE was carried out in ether-narcotized animals. The rats received AA (Acrylamide, extrapure, Helicon) solution in distilled water orally (through a tube) 48, 96, and 144 h after HTE. The material was collected on day 9 after HTE. The following AA doses were studied: 0.496, 2.48, and 12.4 mg/kg, corresponding to 0.004, 0.02, and 0.1 LD_{50} [4].

The preparations were made by a modified method for obtaining isolated TG cells: fixation in formalin, alkaline dissociation for further analysis, and staining with azur and eosin after Romanowskii and Feulgen [1]. The incidence of cytogenetic parameters in TG

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follicular cells (A-cells) was recorded: the percentage of cells with micronuclei, protrusions, internuclear bridges, sum of cytogenetic disorders. In addition, the cytotoxic parameters were recorded: proliferation index (sum of cells with two and more nuclei), nucleus destruction index (percentage of cells with karyopyknosis, karyorrhexis, karyolysis), and apoptotic index. Microscopic analysis of the coded preparations was carried out under an immersion objective at ×1000. A total of 1000 cells from each animal were analyzed. The results were statistically processed using Statistica 5.0 software. The groups were compared by the χ^2 test, the differences were considered significant at $p<0.05$.

RESULTS

No TG cells with micronuclei were detected in intact rats after HTE (control). The relative content of cells with protrusions was 0.83‰, with internuclear bridges 0.33‰, the sum of cytogenetic disorders 1.16‰. The integral proliferation index in intact rats, including TG cells with two and more nuclei, was 13.2‰, apoptotic index 3.5‰ (Table 1).

Exposure to the mutagen in the minimum dose led to maximum increase (8-fold) in the incidence of TG cells with cytogenetic disorders. The incidence of cells with cytogenetic disorders decreased with increasing the mutagen dose (3.6 times higher *vs.* the control) and slightly increased in response to the maximum dose of AA (4.9 times higher *vs.* the control).

The cytogenetic disorders under the effects of all tested mutagen doses were presented by cells with protrusions formed mainly at the expense of aneugenic mechanisms of the division spindle injury [7]. Evaluation of AA effects on the rodent bone marrow and spermatocytes also indicated aneugenic colchicine-like effect of AA [8]. On the other hand, a few micronuclei detected in our study were characterized by small size. This fact presumably indicated that they had formed as a result of DNA aberrations, *i.e.* resulted from the clustogenic effect of AA. Rat TG cell DNA aberrations after oral AA in doses of 2 and 15 mg/kg, approximately corresponding to aberrations found in our study, had also been detected by comet assay [9]. Presumably, the effects of AA were mainly aneugenic, but clustogenic effects on TG cells were also found.

The proliferation and apoptosis parameters in this study are presented by the AA dose–effect curve for TG cells (Fig. 1). A low dose of AA, corresponding to 0.004 LD_{50}, led to a 2-fold increase of the apoptotic index; proliferative activity also increased 2-fold; hence, it seems that cell elimination and restoration in the population were balanced, but this was paralleled by the maximum level of cytogenetic disorders. Increasing the dose by 5 times led to an increase in the apoptotic index (by 4.8 times) in parallel with proliferation inhibition (which reached the control level). This led to a significant elimination of cells with cytogenetic aberrations, which did not recover in the absence of proliferative activity. However, the apoptotic index remained high in response to further increase in AA dose; the total count of cells in the populations presumably decreased, and this fact triggered the compensatory reaction and hence, proliferative activity (proliferation increased by 2.5 times). It is known that increase of proliferation indicates a stronger toxic ef-

TABLE 1. Karyological Parameters (‰) in Rat TG A-Cells in Response to Three AA Doses ($X\pm m$)

Parameter		Relative content of cells with the studied parameter, ‰			
		control	0.496 mg/kg	2.48 mg/kg	12.4 mg/kg
Cytogenetic parameters	Cells with micronuclei	0	0.57±0.30*	0.43±0.20	0.57±0.30*
	Cells with protrusions	0.83±0.48	7.71±1.84***	2.43±0.37*	3.85±1.53***
	Cells with bridges	0.33±0.21	1.14±0.40	1.29±0.52	1.28±0.36
	Sum of cells with cytogenetic disorders	1.16±0.54	9.43±2.23***	4.14±0.40**	5.71±1.710***
Proliferation parameters	Sum of cells with two and more nuclei	13.17±3.24	25.86±5.23***	14.29±2.74	32.43±12.45***
Nucleus destruction parameters	Cells with karyopyknosis	3.5±1.65	7.57±1.39**	17.0±5.32***	16.29±4.98***
	Cells with karyolysis	0	0	0	0
	Cells with karyorrhexis	0	0.14±0.14	0	0.29±0.18
	Apoptotic index	3.50±1.65	7.71±1.43**	17.0±5.32***	16.57±5.04***

Note. Each group consisted of 7 animals. *$p<0.05$, **$p<0.005$, ***$p<0.001$ in comparison with the control (χ^2).

fect of the test substance [2], leading to compensatory reactions and necessitating cell population restoration. This was again paralleled by an increase in the level of cytogenetic injuries (5-fold). Hence, the apoptotic index values, presented mainly by cells with karyopyknosis, were associated with cytogenetic disorders in TG cells, increased with the dose, and reached a plateau. Increase of AA dose was associated with the development of two waves of proliferative activity. The most marked cytogenetic effect of AA was observed in response to the minimum dose, which was presumably because of incomplete triggering of apoptosis.

Hence, a mutagenic effect of AA on TG cells was detected for all the studied doses, which, along with increase in proliferative activity leads to the development of tumors in this organ.

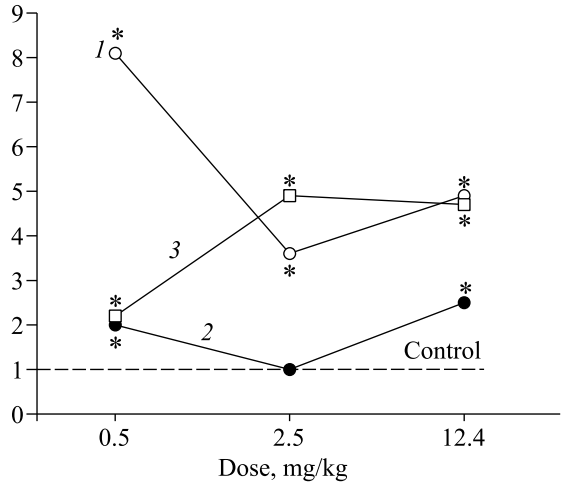

Fig. 1. Karyological parameters of TG A-cells in response to different AA doses. *1*) integral index of cytogenetic disorders; *2*) integral index of proliferation; *3*) apoptotic index. Ordinate: increase of analyzed values (*n*-fold) under the effect of AA *vs*. control level. **p*<0.05 in comparison with the control.

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