Mechanisms of Formation of Micronuclei in Somatic Cells of Tailless Amphibians Normally and under the Effect of N-Nitroso-N-Methylcarbamide

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We studied the relationship between mitotic regimen and incidence of micronuclei in liver and bone marrow cells of tailless amphibians under the effect of genotoxic carcinogen N-nitroso-N-methylcarbamide. Micronuclei appeared in amphibian somatic cells normally and after mutagenic exposure as a result of abnormal mitosis and of "interphase chromatin diminution". This latter variant was most incident in lymphoid cells, presenting as "caudate nuclei", which should be taken into consideration when interpreting findings of micronucleus analysis.

Key Words: *micronuclei; interphase chromatin diminution; somatic mutagenesis; N-nitroso-N-methylcarbamide*

Micronuclear analysis is widely used for the evaluation of cytogenetic injuries in wide-scale screening studies [4,9,12]. However, some aspects of this method remain disputable, *e.g.* formation of micronuclei. According to the most popular viewpoint, micronuclei appear as a result of some forms of abnormal mitotic division, mainly lagging of whole chromosomes and acentric fragments during ana- and metaphases and nonequipolar mitoses [2, 8,11]. It is considered that small micronuclei are formed from lagging acentric fragments, while lagging chromosomes and nonequipolar mitosis yield large micronuclei [4]. Presumably, in this case the incidence of micronuclei can be expected to correlate positively with changed mitotic regimen of the cells. Another viewpoint suggests origination of some micronuclei (irrespective of karyokinesis) through the so-called "interphase chromatin diminution" [7,8]. This mechanism of micronuclei formation was reported for human lymphocytes stimulated with phytohemagglutinin and irradiated *in vitro* [5] and for some other mammalian and human cells [4]. Some scientists claim that micronuclei can form as a result of rrhexis of nuclei with condensed chromatin during apoptotic processes [5]. This hypothesis was not widely accepted, because nuclear fragments forming during apoptosis differ from true micronuclei by their structure and biological role [3].

Tailless amphibians (toads and frogs) are often used for micronucleus analysis; some authors consider them an ideal object for evaluation of mutagenic contamination of the environment by this method [4]. However, the mechanisms of micronuclei formation in somatic cells of these animals remain absolutely not studied. We evaluated correlations between the formation of micronuclei, specific features in the cytological composition and morphology of cell elements, and mitotic activity in hepatic and bone marrow tissues of toads and frogs normally and under the effect of N-nitroso-N-methylcarbamide.

MATERIALS AND METHODS

The study was carried out on *Bufo bufo* toads and *Rana arvalis* frogs. All animals were divided into

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control (n=6) and experimental (n=9) groups. Cytogenetic injuries were induced by N-nitroso-N-methylcarbamide in a dose of 2.5 mg/100 g in 0.64% NaCl injected twice with a 24-h interval [10].

The animals were sacrificed 24 h after the last injection by ether inhalation. Conditions of animal keeping, time of mutagen injection, and sacrifice were identical for all groups (17.00-19.00).

Smears of femoral bone marrow and cytological impressions of the liver were fixed in 96% ethanol and stained by the method of Romanowskii-Giemsa. Micronuclei and mitotic figures were counted in immersion system per 2000 cells. Defective visual fields and mature blood cells (erythrocytes, platelets, and polymorphonuclear leukocytes) were excluded. Pathological forms of mitosis were registered and the percent of lymphoid cells in bone marrow tissue was evaluated.

The results were statistically processed using Statistica software with Student's t test and Spearman's rank correlation coefficient.

RESULTS

The incidence of micronuclei in the liver of frogs and toads increased significantly in experimental groups compared to controls (Table 1). The mitotic coefficient in toad liver also significantly increased. In frogs no significant changes were observed. The incidence of mitoses in the bone marrow decreased significantly in experimental frogs compared to controls; no changes of this kind were detected in toads. The increase in the number of cells with micronuclei in the bone marrow after N-nitroso-N-methylcarbamide injection was significant in toads and negligible in frogs (Table 1). The percent of pathological mitoses in experimental toads reached 20% (*vs.* ≤3% in the control). Pathological forms of cell division included lagging chromosomes and acentric fragments during ana- and metaphases, K-mitoses, and nonequipolar mitoses (processes classically linked with micronucleus formation) [1,2,4,12] (Fig. 1, *a*, *b*).

A strict positive correlation between mitotic activity and incidence of micronuclei was detected in the bone marrow of control toads (r=0.96; p<0.01). In other cases no significant correlation between these parameters was detected (p>0.1). Micronuclei in the bone marrow were much more often seen in frogs than in toads, though in the control this difference was at the level of a trend (p=0.08). The count of lymphoid cells in the bone marrow was 3.5-fold higher in both groups of frogs (p<0.01). A significant positive correlation was detected between lymphocyte count and number of micronuclei in the bone marrow of control toads (r=0.90; p<0.05), while in frogs these changes were at the level of a trend. This relationship seems to be due to greater instability of lymphocyte genome and higher incidence of cytogenetic abnormalities in these cells, including micronuclei [4]. However, no mitotic division of bone marrow lymphocytes was observed in frogs (though in some cases the content of these cells surpassed 90%).

Morphological study of liver and bone marrow cells showed high counts of cells with the so-called "caudate nuclei"; the content of these cells was particularly high among lymphocytes and in some cases surpassed 4%. These formations are sometimes difficult to differentiate from micronuclei [6]. A typical micronucleus of bone marrow plasmablasts and plasma cell in experimental animals was connected to the nucleus with a fine bridge (Fig. 2, *a*, *b*). Caudate nuclei were also detected in other cell types. Despite cytogenetic heterogeneity of this term (origination of caudate nuclei is attributed not only to interphase chromatin diminution, but also to ana- and telophase bridges formed by dicentric chromosomes and to abnormally elongated chromosome arms), many authors acknowledge their role in

TABLE 1. Incidence of Micronuclei, Mitotic Activity of Liver and Bone Marrow Cells of Toads (*Bufo bufo*) and Frogs (*Rana arvalis*) Normally and after Injection of N-Nitroso-N-Methylcarbamide (M±m)

Parameter	Frogs		Toads	
	control	experiment	control	experiment
Bone marrow				
incidence of mitoses, %	0.51±0.17	0.086±0.230*	0.42±0.38	0.46±0.37
incidence of micronuclei, ‰	16.5±9.1	27.5±12.5	8.50±0.47	15.9±0.9**
Liver				
incidence of mitoses, %	0.02±0.04	0.03±0.05	0	0.16±0.13*
incidence of micronuclei, ‰	8.00±0.79	14.80±0.57**	8.34±1.10	16.0±0.6**

Note. *p<0.05, **p<0.01 compared to the control.

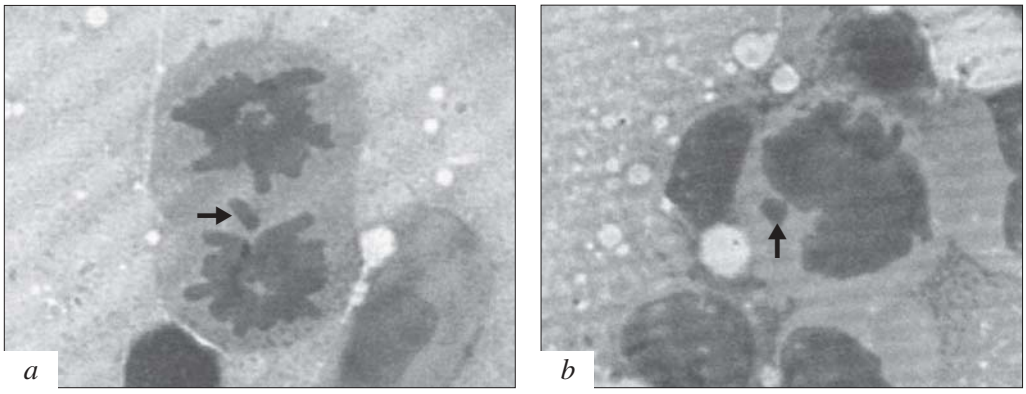

Fig. 1. Mitosis abnormalities in toad bone marrow cells after injection of N-nitroso-N-methylcarbamide. *a*) chromosome lag in anaphase (arrow); *b*) chromosome lag in metaphase (arrow). Here and in Fig. 2: Romanowskii—Giemsa staining, ×900.

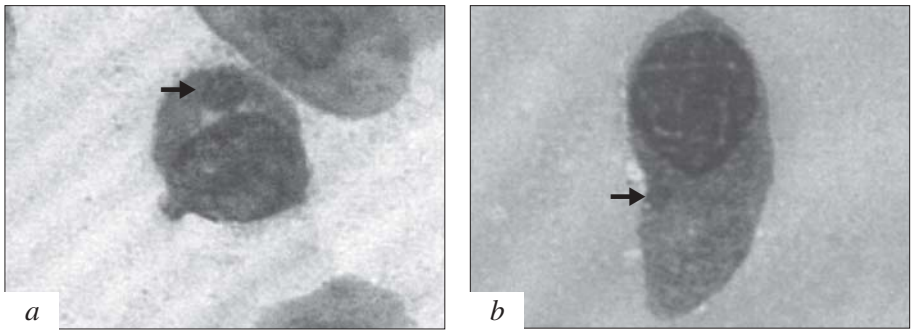

Fig. 2. Chromatin interphase diminution (caudate nuclei) in bone marrow cells of amphibians treated with N-nitroso-N-methylcarbamide. *a*) toad plasmablast; *b*) frog plasma cell. Arrows show caudate nuclei.

the genesis of micronuclei [6]. According to one classification [6], these were caudate nuclei of types 3 and 14 and also of types 1 and 5, whose formation is believed to be linked with interphase chromatin extrusion. Micronuclei (one or several) were often seen in cells together with caudate nucleus.

Hence, the absence of a significant relationship between parameters of mitotic activity in liver and bone marrow cells of experimental toads and frogs of both groups, presence of micronuclei in lymphocytes (their content in some cases being in direct proportion to the micronuclear analysis values) despite complete absence of mitoses in lymphoid cells of experimental frogs and the picture characteristic of caudate nuclei indicate that along with classical karyokinetic mechanism, micronuclei in tailless amphibians can form by the so-called interphase chromatin diminution. This origination of micronuclei is also observed in intact animals, particularly in lymphocytes, but its contribution increases significantly after mutagenic exposure to N-nitroso-N-methylcarbamide, which should be taken into consideration in cytogenetic interpretation of the results of micronucleus test obtained on tailless amphibians.

REFERENCES

1. I. A. Alov, *Cytophysiology and Pathology of Mitosis* [in Russian], Moscow (1972).
2. I. A. Alov and V. V. Kazan'yev, *Dokl. Akad. Nauk SSSR*, **187**, No. 1, 191-192 (1969).
3. Yu. I. Afanas'yev and N. A. Yurina, Eds., *Histology* [in Russian], St. Petersburg (2001).
4. N. N. Il'inskikh, V. V. Novitskii, N. N. Vanchugova, and I. N. Il'inskikh, *Micronuclear Analysis and Cytogenetic Instability* [in Russian], Tomsk (1992).
5. S. N. Kolyubaeva, L. V. Shchedrina, and R. P. Stepanov, *Tsitologiya*, **28**, 227-231 (1986).
6. A. M. Nikiforov, R. F. Fedortseva, E. K. Monosova, *et al.*, *Radiatsion. Biologiya. Radioecologiya*, **40**, No. 3, 299-304 (2000).
7. A. A. Prokof'yeva-Bel'govskaya, *Fundamentals of Human Cytogenetics* [in Russian], Moscow (1969).
8. S. I. Shchelkunov, *Cytological and Histological Analysis of Development of Normal and Malignant Structures* [in Russian], Leningrad (1971).
9. S. B. Dass, S. F. Ali, R. H. Heflich, and D. A. Casciano, *Mutat. Res.*, **381**, No. 1, 243-258 (1997).
10. M. Garriott, C. Piper, and A. Cokkino, *J. Appl. Toxicol.*, **8**, No. 2, 141-144 (1988).
11. J. A. Heddle and A. V. Carrano, *Mutat. Res.*, **44**, 63-70 (1977).
12. S. Sato, M. Taketomi, M. Nakajima, *et al.*, *Ibid.*, **338**, Nos. 1-6, 51-57 (1995).