POSSIBILITY OF USING RAT AND HUMAN PERIPHERAL BLOOD ERYTHROCYTES TO DETECT MUTAGENS AFTER LONG-TERM EXPOSURE

V. S. Zhurkov, E. G. Fel'dt, P. Ressner, and E. L. Skvortsova

UDC 616.155.1-02:575.224.5]-07

KEY WORDS: normochromic erythrocytes; peripheral blood; micronuclei; detection of mutagens

In the early 1980s publications appeared which showed that analysis of erythrocytes with micronuclei in the peripheral blood of mice can be used for the intravital detection of mutagens. High correlation has been demonstrated between the level of normochronic erythrocytes with micronuclei in the peripheral blood and the frequency of cells with chromosomal aberrations in the bone marrow after poisoning of mice for two weeks with cyclophosphamide in the drinking water [2]. Counting micronuclei in the peripheral blood erythrocytes of mice with subacute (2 weeks or more) exposure to chemicals has been shown to be an effective and simple intravital method of detecting mutagens. The possibility of using rat and human peripheral blood erythrocytes to demonstrate mutagenic action has not been studied. We therefore analyzed the frequency of erythrocytes with micronuclei in the peripheral blood of rats and humans exposed to the action of mutagens.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats: 6 animals in the experimental and 6 in the control groups. Benzene in doses of 50, 500, and 1000 mg/kg (0.008, 0.8, and 0.16 LD_{50}) in solution in sunflower oil was injected into rats through a gastric tube for 6 weeks, on 5 days of each week. Peripheral blood films were prepared 6 times, starting from 7 days after the first day of injection. A drop of blood from the caudal vein was placed on a dry defatted slide and a film made. Preparations were dried in air, fixed for 3 min in absolute methanol, and stained by the May—Gruenwald and Giemsa methods. The number of erythrocytes with micronuclei was counted among 2000 erythrocytes. To assess the possibility of using counting erythrocytes with micronuclei in the human peripheral blood as an indicator of the effect of mutagens, blood was analyzed from leukemia patients treated with cytostatics. Blood was taken from a vein. A drop of blood was mixed with a drop of bovine serum on a slide and a film made. Preparations were fixed in absolute methanol and stained by the May—Gruenwald and Giemsa methods. From each subject 1000 normochromic erythrocytes were analyzed. In parallel tests, lymphocyte cultures were set up in two flasks. The duration of culture was 54 and 72 h. Culture of the lymphocytes and preparation of specimens of metaphase chromosomes were carried out in accordance with recommendations in [5]. At each time of culture 100 metaphases were analyzed. Single and paired fragments and chromatid and chromosomal exchanges were counted. No gaps were recorded.

EXPERIMENTAL RESULTS

Analysis of normochromic erythrocytes with micronuclei in blood of rats receiving benzene per orally. In the control animals, at all times of fixation one erythrocyte with a micronucleus was observed in a total number of 72,000

A. N. Sysin Research Institute of General and Communal Hygiene, Academy of Medical Sciences of the USSR, Moscow. Institute of Hygiene and Epidemiology, Prague, Czechoslovakia. (Presented by Academician of the Academy of Medical Sciences of the USSR T. I. Sidorenko.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 9, pp. 248-248, September, 1991. Original article submitted July 9, 1990.

0007-4888/91/0009-1243$12.50

TABLE 1. Results of Cytogenetic Investigation of Patients

Patients' sex	Age, years	Diagnosis	Cytostatics	Number of metaphases with chromosomal aberrations in lymphocyte culture, %		Number of normochromic erythrocytes with micronuclei, %
				54 h	72 h	
F	56	Acute myeloid leukemia	R, A, T	14,0	11,0	0,0
F	25	Acute myeloid leukemia	R, A, T	15,0	13,0	0,0
F	40	Myelodysplastic syndrome	Untreated	—	7,5	0,0
F	57	Chronic myeloid leukemia	R, A, T	—	6,0	0,0
F	34	Chronic myeloid leukemia	R, A, T, C, V	—	16,0	0,0
M	47	Acute lymphatic leukemia	AD, V, A, T, C	12,0	13,0	0,0
M	53	Acute myeloid leukemia	R, A, T	12,0	—	—

Legend. A) Alcysteine; AD) adriablastin; V) vincristine; R) rubidomycin; T) thioguanine; C) cyclophosphamide.

erythrocytes (frequency $1.4 \cdot 10^{-5}$). After exposure to benzene (50 mg/kg) no erythrocytes with micronuclei could be seen. In groups of animals receiving benzene in doses of 500 and 1000 mg/kg fluctuations in the frequency of erythrocytes with micronuclei were within the control limits. Altogether in the experimental and control groups there were seven cases when one erythrocyte with a micronucleus was found among 2000 erythrocytes. The total frequency of erythrocytes with micronuclei, according to data for all groups (among 288,000 erythrocytes there were only seven with micronuclei) was $2.4 \cdot 10^{-5}$.

Thus after peroral administration of benzene we found no accumulation of erythrocytes with micronuclei in the peripheral blood of rats. Meanwhile, in the case of subcutaneous injection and inhalation of benzene, the frequency of cells with chromosomal aberrations in the bone marrow of rats was increased [1, 4]. In experiments on mice, benzene increased the frequency of cells with chromosomal aberrations and of polychromatophilic erythrocytes with micronuclei in the bone marrow [6] and of normochromic erythrocytes with micronuclei in the peripheral blood [9].

The absence of an effect in the rats may be connected with selective elimination of erythrocytes with micronuclei from the peripheral blood. Treatment of rats with a mutagen has been shown to increase the frequency of erythrocytes with micronuclei in the peripheral blood only of splenectomized animals [12]. In mice, no such mechanism of selective elimination of defective erythrocytes by the spleen is present, as has been shown by experiments with benzene [8].

Analysis of Normochromic Erythrocytes with Micronuclei in Human Peripheral Blood. The results of the cytogenetic study of the patients are given in Table 1. No erythrocytes with micronuclei were found in any single case. Meanwhile the frequency of metaphases with chromosomal aberrations in the lymphocyte cultures from patients varied from 6.0 to 16.0%, significantly more than the spontaneous level of this parameter in healthy blood donors, which, as several workers have shown, in 1-2%. We also analyzed blood films from a healthy donor. Among 20,000 erythrocytes there were two with micronuclei (frequency $1 \cdot 10^{-4}$).

The results of cytogenetic analysis of lymphocytes indicate a significant mutagenic effect of the cytostatics in the patients. Similar results were obtained in many investigations. In the bone marrow of patients with leukemia, treated with cytostatics, the frequency of polychromatophilic erythrocytes with micronuclei was increased [7, 10]. Meanwhile, we found no increase in the frequency of erythrocytes with micronuclei in the peripheral blood of patients in whom a significant increase in peripheral blood lymphocytes with chromosomal aberrations was observed.

The results of other investigations into the possibility of analyzing erythrocytes with micronuclei in human peripheral blood as an indication of mutagenic action are interesting. In a study of 81 individuals aged from 20 to 34 years the average frequency of erythrocytes with micronuclei in the blood of oil workers was $(2.1 \pm 0.2) \cdot 10^{-4}$, whereas in subjects not working in industry it was $(1.6 \pm 0.2) \cdot 10^{-4}$ [3]. Correlation was found between this parameter and the immunoreactivity of the individual. In a study of 125 persons after splenectomy or with total absence of splenic function, the average frequency of RNA-positive erythrocytes with micronuclei was 3.3 per thousand, and the number of normochromic erythrocytes with mironuclei was 2.7 per 1000 [11, 13]. The authors cited observed correlation between exposure to environmental factors and the frequency of erythrocytes with micronuclei in splenectomized patients.

It will be clear from these results that in man, just as in rats, intensive selection of erythrocytes with micronuclei takes place in the spleen, as the following facts show: 1) a significantly lower spontaneous level of erythrocytes with micronuclei in human ($1\text{-}3 \cdot 10^{-4}$) and rat ($2.4 \cdot 10^{-5}$) blood compared with the frequency of polychromatophilic erythrocytes with micronuclei in the bone marrow ($1\text{-}2 \cdot 10^{-3}$); 2) an increase in the frequency of erythrocytes with micronuclei in human and rat blood after splenectomy, up to the level observed in bone marrow erythrocytes; 3) absence of any increase

in the frequency of erythrocytes with micronuclei in rats after exposure to mutagens and in man following treatment with cytostatics; 4) detection of a significant effect of mutagens during analysis of erythrocytes with micronuclei in splenectomized rats and humans.

The results described above thus point to the possibility of using counting micronuclei in peripheral blood erythrocytes as a way of detecting mutagens, but only in experiments on mice; nevertheless, this is of definite value in practical toxicology, for with this intravital method it is possible to observe the cytogenetic effect of the test factors over a period of time.

LITERATURE CITED

1. V. B. Dobrokhotov and M. I. Enikeev, Gig. San., No. 1, 32 (1977).
2. V. S. Zhurkov, P. Ressner, A. Pastorkova, and E. G. Fel'dt, Byull. Éksp. Biol. Med., No. 8, 222 (1986).
3. N. N. Il'inskikh, M. A. Medvedev, G. V. Potapova, et al., Gig. San., No. 12, 18 (1989).
4. A. A. Lyapkalo, Gig. Truda, No. 3, 24 (1973).
5. "A Method of Counting Chromosomal Aberrations as a Biological Indicator of the Influence of External Environmental Factors on Man," Technical Recommendations [in Russian], Moscow (1974).
6. E. G. Fel'dt, Gig. San., No. 7, 21 (1985).
7. T. Abe, T. Isemura, and J. Kikuchi, Mutat. Res., **130**, No. 2, 113 (1984).
8. R. Barale, F. Giorgelli, L. Megliore, et al., Mutat. Res., **144**, No. 3, 193 (1985).
9. W. N. Choy, J. T. MacGregor, M. D. Shelby, and R. R. Maronpot, Mutat. Res., **143**, No. 1, 55 (1985).
10. B. Höstedt, et al., Cancer Genet. Cytogenet., **3**, No. 3, 185 (1981).
11. J. T. MacGregor, Environ. Molec. Mutagen., **14**, Suppl. 121 (1989).
12. R. Schlegel and J. T. MacGregor, Mutat. Res., **127**, No. 2, 169 (1984).
13. D. Smith, J. T. MacGregor, K. Hooper, et al., Environ. Molec. Mutagen., **11**, Suppl 11, 97 (1988).

COMBINED EFFECT OF DESIALATED AND GLYCOSYLATED LOW-DENSITY LIPOPROTEINS ON LIPID ACCUMULATION IN INTIMAL CELLS OF THE HUMAN AORTA IN VITRO

I. A. Sobenin, V. V. Tertov, and A. N. Orekhov

UDC 612.015+616.132+612.397.81

KEY WORDS: atherosclerosis; diabetes mellitus; desialated low-density lipoproteins; glycosylated low-density lipoproteins; lipid accumulation.

Diabetes mellitus leads to the earlier onset and more rapid progression of atherosclerosis [4]. The mechanism of enhanced atherogenesis in diabetes has not yet been explained. A characteristic feather of atherosclerosis is massive deposition of lipids in cells of the vascular wall. It has recently been shown that the blood sera of most patients with coronary atherosclerosis and also blood sera of diabetics can induce cholesterol accumulation in cultures of cells taken from the intact intima of the human aorta [1, 11]. The atherogenic effect of sera from patients with atherosclerosis has been shown to be due mainly to low-density lipoproteins (LDL) [12], which differ from healthy human LDL in their low sialic

Institute of Experimental Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Smirnov.)

Translated from Byulleten Éksperimental'noi Biologii i Meditsiny, Vol. 112, No. 9, pp. 250-252, September, 1991. Original article submitted July 19, 1990.