Spontaneous DNA Damage in Peripheral Blood Leukocytes from Donors of Different Age

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Spontaneous DNA damage in peripheral blood cells was studied in healthy donors of different age (23-70 years). Alkaline comet assay was used to evaluate total DNA damage in individual cells. The individual variability in venous blood samples was higher than in capillary blood samples. The advantage of analysis of DNA damage in nucleated cells from the whole blood is more preferable compared to experiments with isolated lymphocytes because all cell populations in the sample are analyzed. Study of blood cells from healthy donors showed that the mean percent of DNA in the comet tail tended to decrease with age. However, correlation analysis revealed no relationship was found between donor age and degree of spontaneous DNA damage.

Key Words: *comet assay; DNA damage; age*

DNA damage is a biological marker of such processes as oxidative stress, DNA reparation, and cell death. Study of DNA damage holds promise for epidemiological, genotoxicological, sanitary, and hygienic examinations, diagnostics, and monitoring of disease therapy and prevention [1,8]. Spontaneous DNA damage in living cells depends on the diet, exposure to stress factors, environmental conditions, and age. Single-cell gel electrophoresis (comet assay) is most adequate to study DNA damage in nonproliferating cells. This method allows evaluation of DNA damage in individual nucleated cells [9].

Comet assay showed higher levels of spontaneous DNA damage in peripheral blood cells from patients with diabetes and breast cancer compared to healthy donors [3,6]. The degree of DNA damage is estimated from a computational study of nucleoid microimages for the comet length, comet tail length, comet tail moment, and percentage of DNA in the comet tail (TDNA) [9].

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Spontaneous DNA damage is a result of normal metabolic processes in DNA and various genotoxic exposures, including attack of reactive oxygen species (*e.g.*, during their intracellular generation), changes in the antioxidant state, spontaneous apurinization/apyrimidinization of DNA, and activity of intracellular enzyme systems. However, there are conflicting data on age-related variations in DNA damage. Moreover, comet assay produces different results on DNA damage in blood cells from healthy donors [7,8,10]. These facts are probably associated with individual variability and differences in experimental conditions. Further systematic study of spontaneous (basal) DNA damage should be performed with leukocytes from healthy donors of the same age under similar experimental conditions.

This work was designed to estimate basal DNA damage in peripheral blood cells from healthy donors. DNA damage was studied in 48 donors of different age.

MATERIALS AND METHODS

Whole blood samples were obtained at the outpatient department of the Pushchino Research Center

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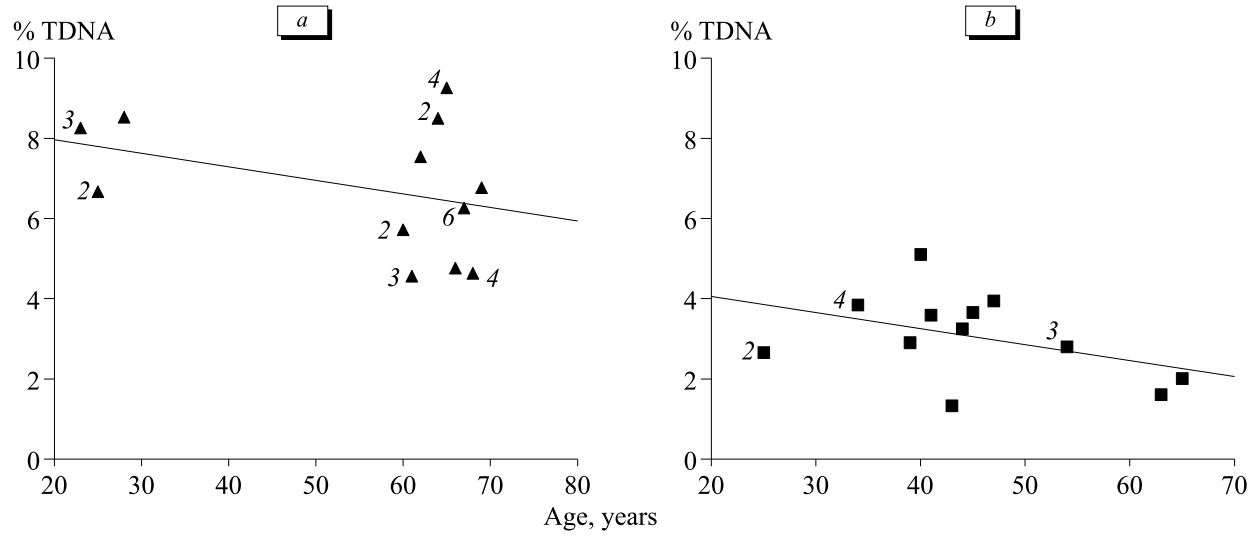

Fig. 1. Dependence of basal DNA damage in leukocytes on the age of donors. Whole venous blood (*a*); finger blood (*b*). Numerals near points in the chart designate the number of patients of the same age. Points without numerals present the data for 1 patient.

(Program "Fundamental Sciences to Medicine", 2006). Blood aliquots from finger or vein of 48 healthy donors (23-70 years) were placed in tubes with K-EDTA. The test preparations were obtained from lymphocytes and whole blood (5-fold dilution with phosphate buffered saline). Lymphocytes were isolated using Ficoll-verografin [2].

Agarose (1%) was layered onto a glass slide. The samples were diluted in EDTA-phosphate buffer, mixed with an equal volume of 1% low-melting-point agarose (37°C), and applied to a solidified agarose layer. After solidification, cell-containing agarose was covered with a layer of 0.5% low-melting-point agarose. These slides were maintained in a lysing solution of 1% sodium sarcosinate, 2.5 M NaCl, 0.1 M EDTA, 0.01 M Tris-HCl (pH 10), and 1% Triton X-100 at 4°C for 1 h. Immediately after lysis, the slides were put in alkaline solution A (0.3 M NaOH and 0.001 M EDTA, pH>13) for 20 min. After alkaline treatment, the slides were transferred to a SE-1/S-1N electrophoretic chamber (Khelikon Company) and subjected to electrophoresis in a freshly prepared portion of solution A at 4°C for 20 min (buffer volume 250 ml, voltage 27 V, current strength 260-270 mA, electric field strength 2 V/cm). After electrophoresis, the slides were washed with distilled water and stained in phosphate buffered saline containing 2 μg/ml ethidium bromide for at least 1 h. The samples were examined under a LYuMAM I-3 fluorescence microscope (LOMO). The images were obtained with a Nikon CoolPix 995 digital camera and analyzed using special software for the calculation of standard parameters of comets [4]. TDNA was measured. The greater was the value of TDNA, the higher was the degree of DNA damage.

Three slides were prepared for each blood sample. We photographed 30-40 cells in each slide.

The results were analyzed by Student's test ($p<0.05$) and Pearson correlation test.

RESULTS

TDNA for the venous blood significantly differed between young (23-28 years) and elderly donors (66-69 years, $p=0.039$). The mean TDNA in these donors was 7.8±0.6 and 5.6±0.5%, respectively. Similar results were obtained for cells of the venous (Fig. 1, *a*) and capillary blood (Fig. 1, *b*). The linear regression curve for TDNA decreased with age. However, correlation analysis revealed no relationship between donor age and degree of spontaneous

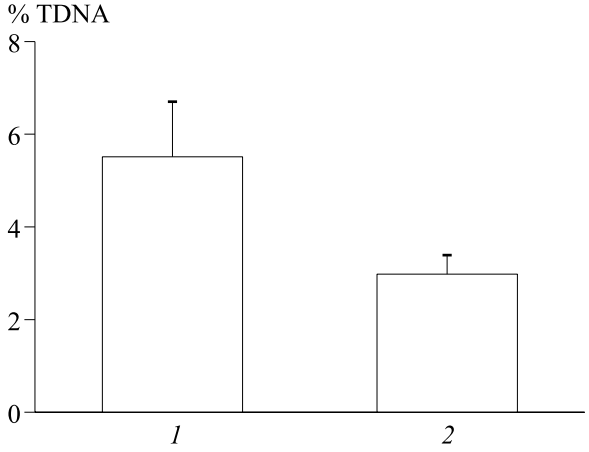

Fig. 2. Basal DNA damage after incubation of samples in RPMI 1640 medium. No incubation (*1*); incubation of slides at 37°C for 60 min. The venous blood was taken from 6 elderly donors of 61-68 years.

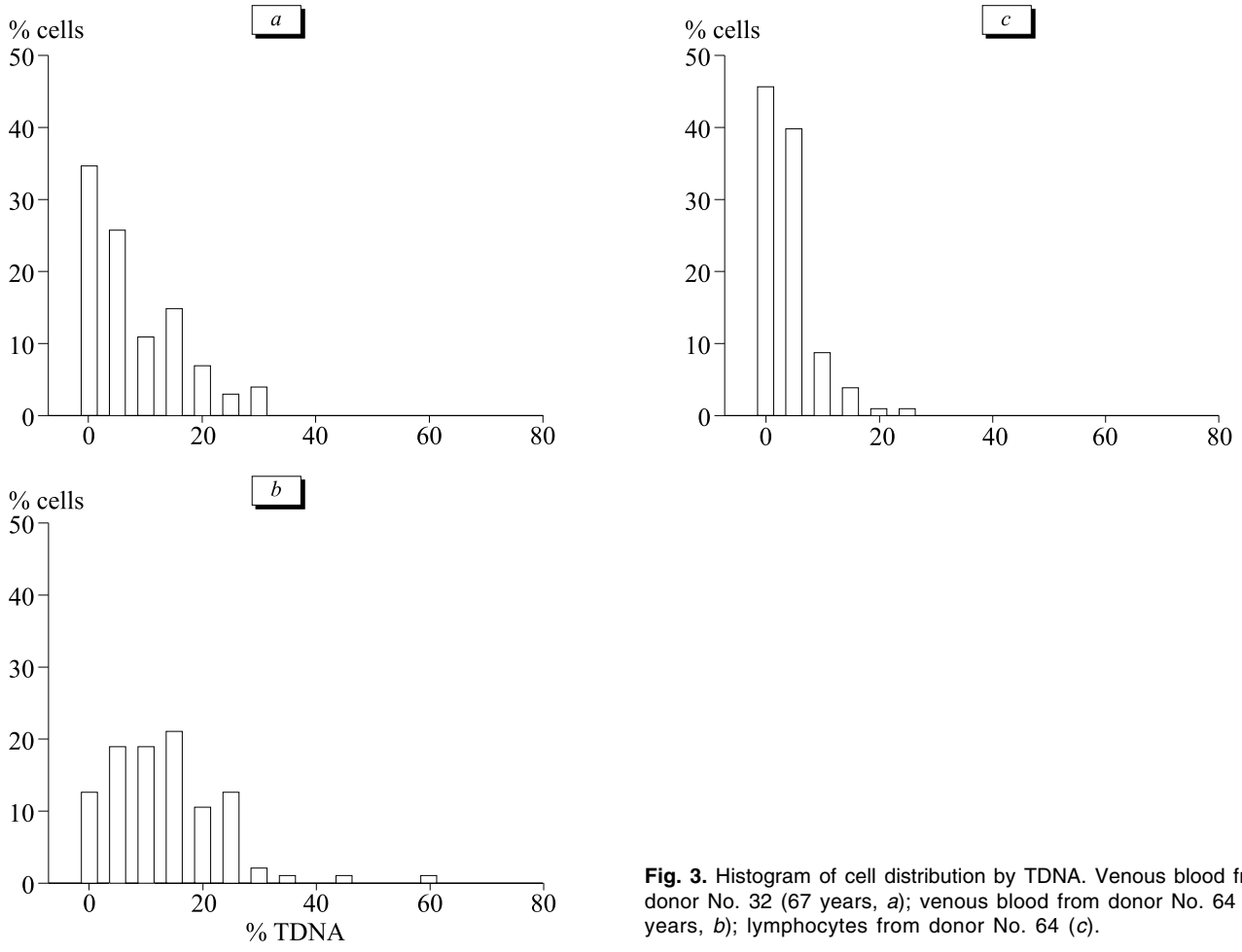

Fig. 3. Histogram of cell distribution by TDNA. Venous blood from donor No. 32 (67 years, *a*); venous blood from donor No. 64 (23 years, *b*); lymphocytes from donor No. 64 (*c*).

DNA damage (r=-0.42). Our previous studies produced the same results [10]. Significant interindividual variations were revealed in the mean TDNA. The degree of DNA damage in venous blood samples was higher than in capillary blood samples (Fig. 1). The mean TDNA in the venous and capillary blood was 6.8±0.5 and 3.1±0.3%, respectively ($p \leq 0.001$). It was probably associated with differences in DNA damage due to apurinization/apyrimidinization in cells of the venous and capillary blood and/or various temperature conditions of alkaline electrophoresis. The average temperature of the solution over slides after electrophoresis of the venous and capillary blood was 14.8 (9-18°C) and 8.6°C (7-10°C), respectively. Incubation of slides with venous blood cells in serum-free RPMI 1640 medium at 37°C for 60 min decreased the degree of DNA damage (TDNA 2.98± 0.4%, p=0.044), which became comparable to that for capillary blood cells (TDNA 3.09±0.35%; Fig. 2).

The distribution of cells by DNA damage was studied in whole blood samples from elderly (No. 32, 67 years; Fig. 3, *a*) and young donors (No. 64, 23 years; Fig. 3, *b*) and isolated blood lymphocytes from the same donor (Fig. 3, *c*). The young donor had high average value of TDNA (10.08±1.01) and contained cells with high degree of DNA damage (TDNA≥20 in 10% cells). The cells with a DNA damage of more than 40% were attributed to apoptotic cells [1,5,11]. The ratio of isolated lymphocytes with DNA damage was low. Apoptotic lymphocytes were not identified. These specific features are probably related to the method of cell isolation, since purification on Ficoll-verografin results in the elimination of damaged cells [2]. The histogram of cell distribution shows that the ratio of cells with DNA damage is low in the elderly donor.

Comet assay shows that TDNA of blood cells from healthy donors tended to decrease with age. The advantage of studying the DNA damage response in nucleated cells from whole blood over the experiment with isolated lymphocytes is the involvement of all cell populations in a sample. The variability in venous blood samples is higher than in capillary blood samples. We conclude that age-

related characteristics should be taken into account during analysis of basal DNA damage in blood cells from healthy donors and patients with various diseases.

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