ONCOLOGY

Deoxyribonuclease Activity in Biological Fluids of Healthy Donors and Cancer Patients

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 146, No. 7, pp. 97-100, July, 2008
Original article submitted August 3, 2007

Integral activity of neutral deoxyribonucleases in the plasma and urine or donors, patients with benign prostatic hyperplasia, and patients with stomach and prostatic cancer was studied by IFA based on hydrolysis of DNA fragment modified with haptene molecules. In donors plasma deoxyribonuclease activity was 0.16±0.04, urinary activity 1.49±1.41 act. U/ml. In patients with benign prostatic hyperplasia and malignant tumors the integral activity of blood deoxyribonucleases was significantly below the normal, and in tumors it did not correlate with tumor size and disease stage. A significant correlation between blood and urinary deoxyribonuclease activities was detected.

Key Words: *blood plasma; urine; deoxyribonuclease activity; prostatic cancer; stomach cancer*

Exogenous DNA rapidly degrade in the blood [11], which attests to high activity of blood nucleases and seems to be one of the mechanisms for protection from foreign nucleic acids [1]. The major DNA-hydrolyzing enzyme in the blood is neutral DNase I, though in addition to this enzyme, acid DNase II, phosphodiesterase I, and other proteins, hydrolyzing nucleic acids, circulate in the blood [4]. Apart from DNases, their inhibitors, the main of which is actin [4], were found in the blood stream, and hence, DNase activities are determined by the proportion between the concentration of DNasse and their inhibitors. Endogenous circulating nucleic acids are always present in the blood stream of normal subjects; they appear as a result of apoptosis and necrosis [9] and their low concentrations can result from rapid hydrolysis under the effects of nucleases [12]. Plasma concentrations of these DNA in patients with cancer and autoimmune diseases are as a rule several-fold higher than in donors [2,3]. The blood of patients with systemic lupus erythematosus and rheumatoid arthritis contains, in addition to circulating DNA, antibodies to DNA [10] induced by circulating DNA complexes with histones and DNA-binding proteins [5]. It was shown that activity of DNase I in the blood of patients with systemic lupus erythematosus was lower than in donors, which presumably disorders clearance of circulating DNA. Hence, blood DNases are presumably involved in the pathogenesis of cancer and autoimmune diseases.

Modern data on activity and concentrations of blood DNases in health and disease are scanty and often contradictory [4], presumably because of insufficient attention to measurements of these en-

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0007-4888/08/1461-0089

zyme activities and use of different methodological approaches precluding correct results.

We measured DNase activities in the blood and urine of donors and cancer patients by IFA based on hydrolysis of a DNA fragment modified with biotin and fluorescein molecules.

MATERIALS AND METHODS

Blood specimens of healthy men (n=30) and women (n=30) were obtained from Central Clinical Hospital, Siberian Division of Russian Academy of Sciences. Blood specimens of primary patients with benign prostatic hyperplasia (BPH; n=22) and prostatic cancer (n=5) were collected at Municipal Clinical Hospital No. 1, of patients with stomach cancer (n=15) at Novosibirsk Oncological Center. The studies were carried out with consideration for the patients' free will and privacy principles in accordance with Basic Legislation of the Russian Federation on Public Health Protection (Order of the President of the Russian Federation No. 2288 of December 24, 1993). The stage of the disease was determined according to TNM classification.

Blood was collected in 0.05 M EDTA solution in 10 mM Tris-HCl, 0.15 M NaCl (pH 7.5), with blood:EDTA proportion of 1:5. Blood specimens were stored at 4°C and processed within 6 h after collection. Blood cells were precipitated by centrifugation at 450g for 15 min, after which the supernatant was collected and centrifuged for 15 min at 1500g. The plasma was collected and stored in aliquots at -20°C.

Specimens of morning urine were processed within 20 min after collection. Urinary cells were precipitated by centrifugation at 550g for 20 min, the resultant supernatant was stored in aliquots at -20°C.

Integral activity of DNases was evaluated by IFA based on hydrolysis of a PCR fragment of DNA modified by fluorescein and biotin residues [6]. In order to plot the calibration curve, the PCR fragment adsorbed on avidin-treated wells of polystyrene plates was incubated with serial dilutions (0.005-0.500 U/ml) of DNase I (Fermentas). Unhydrolyzed DNA fragments were detected by specific antibodies to fluorescein with subsequent immunoperoxidase detection of immune complexes. The sensitivity of the method in DNase I activity units was 0.05 U/ml sample, with coefficient of variations for each point below 4%. The levels of DNase activities in biological fluids were evaluated in samples of 10 μl for plasma and 2 μl for urine.

The results were processed using Statistica 6.0 software with the use of parametric (Student's, χ^2 tests) and nonparametric (Mann—Whitney, Spearman coefficient of correlations) methods.

RESULTS

The distribution of integral DNase activity in donor plasma is normal (χ^2 test, p<0.05). Its mean level was 0.16±0.04 DNase I U/ml blood (p<0.05; Fig. 1, *a*) (0.13±0.04 DNase I U/ml blood in men (Fig. 1, *b*) and 0.20±0.06 DNase I U/ml blood in women). Comparison of these samples using Student's test showed no statistically significant differences in the levels of DNases integral activities in the blood of men and women.

Our data are in line with the findings of other authors, measuring serum DNase activity by spectrophotometric analysis [7].

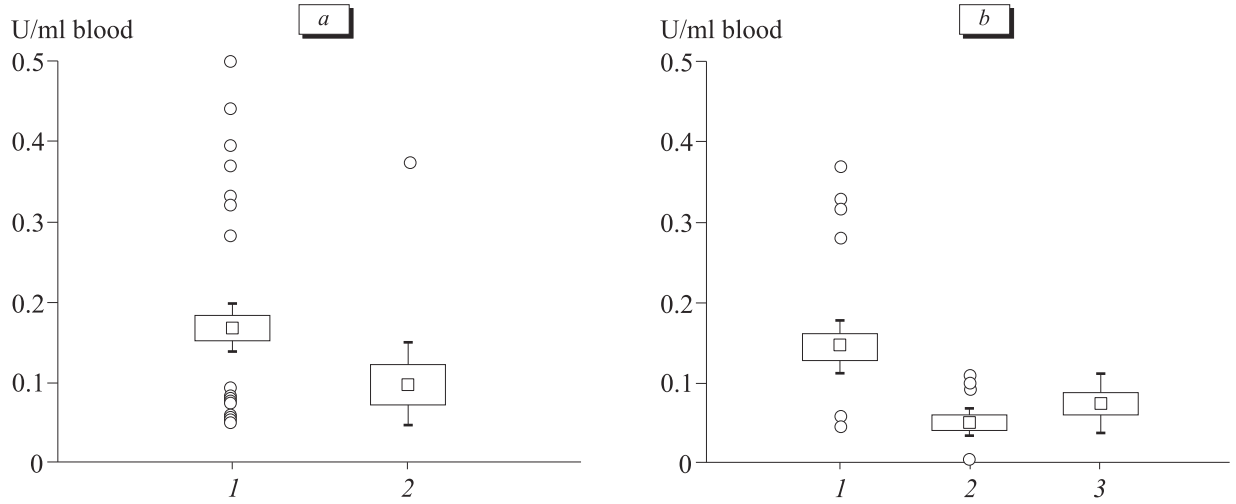

Fig. 1. Blood DNase activity. *a*) donors (*1*) and patients with stomach cancer (*2*); *b*) healthy men (*1*), patients with BPH (*2*) and prostatic cancer (*3*).

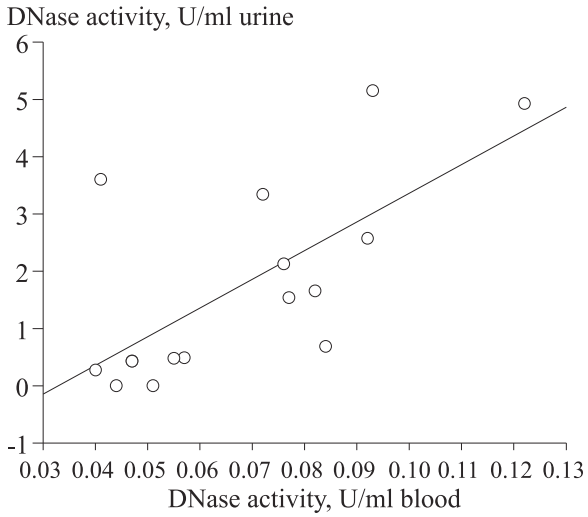

Fig. 2. Relationship between blood and urinary DNase activities in patients with BPH and prostatic cancer.

In cancer patients, integral DNase activity in the blood was below the detection level in 21% cases, being 0.10 (0-0.37) DNase I U/ml blood in stomach cancer (Fig. 1, *a*) and 0.07 (0.05-0.12) DNase I U/ml blood in prostatic cancer (Fig. 1, *b*). In BPH patients the level of plasma integral DNase activity was also below the normal (0.05 (0-0.18) DNase I U/ml blood). Comparison of integral plasma DNase activities in donors, patients with cancer and BPH showed that disease development was associated with reduction of DNase activity (Mann—Whitney test; $p<0.01$). No correlation between DNA activity and cancer stage was detected.

The levels of urinary integral DNase activity in donors did not depend on sex, its mean value being 1.31±0.65 DNase I U/ml blood ($p<0.05$; 1.01±0.48 DNase I U/ml blood in men and 1.92±1.38 DNase I U/ml blood in women). It was shown that urinary DNase activity in healthy men did not differ from that in patients with prostatic diseases (1.92±0.97 and 1.32±1.31 DNase I U/ml blood ($p<0.05$) in BPH and malignant tumors, respectively), but correlated with its level in the blood ($r=0.67$; $p<0.05$), which suggests infiltration of the main part of DNases into the urine from the blood (Fig. 2).

Low activity of DNases in the blood of cancer patients can be a factor leading to an increase in circulating DNA concentration in the blood. The pathogenetic role of lasting circulation of considerable levels of DNA in the blood of cancer patients is unknown, but presumably they can induce pathogenetic processes similar to those in patients with autoimmune diseases. In addition, experimental data confirm the genometastasis theory [8] and hence, circulating DNA can be involved in tumor metastasizing. Irrespective of the role in the pathogenesis, high concentration of circulating DNA in the blood suggests using the blood as a source of oncospecific material in PCR diagnosis of tumors.

The causes of reduction of integral activity of DNases in the blood of cancer patients in comparison with donors was not studied. Presumably, tumor development is associated with elevation of DNase inhibitor concentrations in the blood [7], which can lead to reduction of nuclease activity. In addition, high concentration of circulating blood DNA can lead to reduction of DNase activities.

As the integral activity of blood and urinary DNases varies greatly even within the same group, it can hardly be used as a marker for the diagnosis of cancer. However, detection of the role of blood DNases in the pathogenesis of cancer and autoimmune diseases will presumably lead to the use of integral DNase activity for more accurate diagnosis and choice of optimal treatment protocol.

The study was supported by the Russian Foundation for Basic Research (grant No. 06-04-49732a), grants of President of the Russian Federation (MK-59.2007.4), Basic Research in Higher Education (No. Y4-B-08-13), Russian Ministry of Education (RNP.2.2.2.3.10035), Integration project of Siberian Division of Russian Academy of Sciences No. 13, and Foundation for National Science Promotion.

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