Structural Changes in Blast Cell Chromatin and DNA in Patients with Acute Leukemia After Cytosar and Irradiation Treatment

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It is known that chromatin DNA is the main target for many drugs in the living cell. Drugs which bind with double helix DNA are divided into two classes:

1) those which directly interact with DNA and change its stability and conformation and
2) those which indirectly influence DNA.

Cytosar (or β-cytosine arabinoside) belongs to the first class of drugs. Influence of Cytosar on DNA and RNA is not restricted only to its binding with these structures. It also makes ruptures in double helix DNA, causing cell destruction [1].

Here we present microcalorimetrical results, showing how Cytosar and laser irradiation influence the structure stability of leukocyte chromatin in vivo and leukocyte DNA in solutions. We think that these results lead to a better understanding of how genetic material behaves in vivo under the influence of chemical and physical factors of cells.

A differential scanning microcalorimeter was used to study the complex biological systems [2]. Its sensitivity is 10^{-7} W, the measuring cell volume 0.2 cm^3, and heat rate 20 °C/h. Irradiation was carried out with two beams of helium-neon laser (30 mW) for 10–15 min. Twenty samples were studied. Cytosar was added so that each DNA nucleotide pair had 0.1 ± 0.01 of a molecule. Cells were incubated at 37 °C for 1 h.

In earlier works it was shown that the thermal denaturation process of cell suspension can be divided into three main temperature ranges: the first range 40°–65 °C with $T_d^1 = 57$ °C, the second 62°–75 °C with $T_d^2 = 73$ °C, and the third 75°–95 °C with $T_d^3 = 82$ °C. It was established that in the first and second ranges membranes, proteins, and ribonucleic protein (RNP) complex were denatured but in the third, the chromatin complex was denatured [3].

Figure 1 shows heat absorption curves of normal cells, leukocytes, and blast cells of human bone marrow. In the case of pathology, the chromatin complex in cell composition is more thermostabile than the norm. The temperature corresponding to the maximum blast cell chromatin denaturation peak is ~ 87 °C. This value is 7 °C higher than in the norm ($T_d = 80$ °C). Striking changes are observed in other parameters too: T_d increases by 4°–11 °C (in the norm $\Delta T_d = 7$ °C), melting enthalpy (Q_d) increases by 30%, i.e., 26.0 ± 3.0 kal/g DNA. As for thermostability of leukocyte RNP complex it is also more thermostabile than the norm by approx. 4 °C.

During incubation of the blast cells with the cytostatic drug Cytosar, we observe a shift of the chromatin peak maximum (Fig. 1, curve 3) to a temperature 3 °C lower in respect to leukocytes ($T_d = 84$ °C) and the peak narrowing to $\Delta T_d = 9.5$ °C. The analogical situation is observed for RNP complex too. After laser irradiation of blast cell suspension previously incubated with Cytosar for 12 min, the thermodynamic characteristics of chromatin denaturation and

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RNP complex processes become more similar to the norm parameters.

From these results we can conclude that Cytosar and laser irradiation in used doses destabilize the tumor chromatin complex, bringing its thermodynamic parameters closer to the norm.

Figure 2 presents heat absorption curves of human bone marrow leukoblasts after laser irradiation for 12 and 15 min. In the first case (curve 1), chromatin T_d is shifted to a lower temperature by 7 °C for leukocytes but the width of the peak half height is narrowed by 3 °C. Q_d remains almost unchanged, i.e., 26.5 ± 3.0 kal/g DNA. We also observed other changes: In particular, a clear shoulder has appeared on the high temperature side at $T_d = 87$ °C. At 15 min irradiation $T_d = 75$ °C for leukocytes this value is 12 °C less; $\Delta T_d = 9°$ and $Q_d = 26.2 \pm 3.0$ kal/g DNA. In this case we again observe a high temperature shoulder at 87 °C.

From the results given above, it follows that after laser irradiation of intact blast suspension chromatin complex the thermostability decreases by 5°–12 °C, but after irradiation of suspension previously incubated with Cytosar, the denaturation temperature decreases by 7 °C (Fig. 1, curve 4). It points to the rough structural disturbances in chromatin complex. Consequently, laser irradiation influences the effect of cytostatic drug preparations on blast cell genome models in vitro.

It was interesting to find out what caused T_d decrease of leukocyte chromatin in cell composition after treatment with Cytosar and laser irradiation. Its decrease was probably connected with the decrease in DNA double helix thermostability as a result of its structure change or with change of the histone binding strength or histone proteins with leukocyte chromatin DNA.

As seen in Fig. 3, addition of Cytosar in leukocyte DNA solution causes a shift in the melting curve to higher temperatures (T_d), a broadening of the transition interval (ΔT_d) and an increase of the melting enthalpy (Q_d) by 1.5 °C, 2.5 °C,

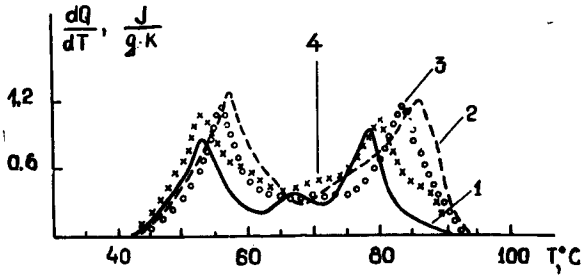

Fig. 1. Heat absorption curves of human bone marrow cells in Henks' solution (pH 7.2). *1*, Norm; *2*, leukocytes; *3*, blast cells treated with Cytosar ($r = 0.01$ mol cyt./bp DNA); *4*, blast cells treated with Cytosar after 12 min laser irradiation

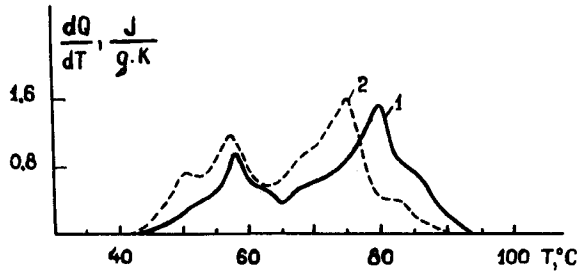

Fig. 2. Heat absorption curves of human bone marrow blast cells in Henks' solution (pH 7.2). *1*, after 12 min laser irradiation; *2*, after 15 min laser irradiation

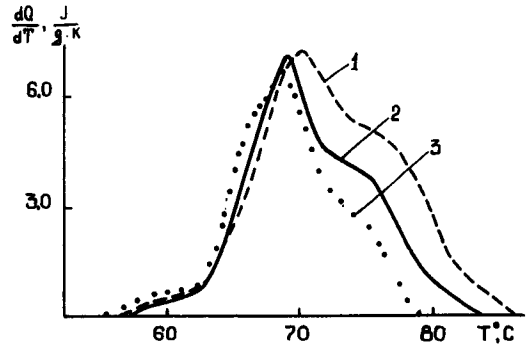

Fig. 3. Heat absorption curves of DNA dilute solutions. *1*, Norm; *2*, DNA + Cytosar ($r = 0.01$ mol.cyt/bp DNA); *3*, DNA + 12 min laser irradiation ($C_{DNA} = 0.12\%$, pH 7.2, 0.1 SSC)

4.5 kal/g DNA, accordingly. Besides this, a strong shift (by ~ 3 °C) to higher temperatures of a high temperature shoulder ($T_d \sim 77$ °C) by 1 °C in comparison with the main fraction ($T_d \sim 69$ °C) is clearly seen. We think that observed changes of the transition parameters (Q_d, ΔT_d, T_d)

and melting curve profile are connected with the fact that Cytosar weakly but specifically stabilizes GC pairs.

So we see that the influence of Cytosar on the DNA molecule is not the same in vivo and in vitro. Nearly the same situation is observed in the case of laser irradiation.

We conclude:

1) Cytosar in its metabolized form acquires new properties leading to disturbance of double helix DNA structure [1];
2) it influences genetic material through change of interaction between DNA, histones, and nonhistone proteins.

So these data show that study of DNA physicochemical properties alone is not always enough to understand DNA behavior in vivo. We can also affirm that the method of scanning microcalorimetry developed in the Institute of Physics of Georgian Academy of Sciences can serve as a convenient and cheap method but in some cases as an quick method for discovering drug influence on genetic material in vivo.

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