ENZYME THERMOSENSITIVITY OF LEUKOCYTES WITH TRISOMY FOR CHROMOSOME 21

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The thermosensitivity of glucose-6-phosphate dehydrogenase and myeloperoxidase was studied in lysates of leukocytes from patients with Down's syndrome. A statistically significant increase in temperature sensitivity of these two enzymes was discovered in leukocytes trisomic for chromosome 21 compared with the control. The differences observed may be based on an increased frequency of mutation injuries in the aberrant cells and also of abnormal post-translation modification of the protein molecules.

KEY WORDS: Down's syndrome; leukocytes; temperature sensitivity of enzymes.

An important problem in clinical genetics is the study of molecular-biochemical processes of realization of genetic information in aneuploid cells and, on that basis, the creation of an integral concept of the pathogenetic mechanisms of development of the abnormal phenotype in the presence of chromosomal imbalance.

A general symptom characteristic to a greater or lesser degree of virtually all patients with chromosomal diseases is the development of signs of premature aging. This is manifested particularly clearly in Down's syndrome [1].

In the process of aging, according to Orgel's theory [12], mutant proteins with modified physicochemical structures accumulate in the cells. One characteristic feature of the physicochemical state of the protein molecule is its temperature sensitivity.

The object of this investigation was to study the temperature sensitivity of glucose-6-phosphate dehydrogenase (G6PD) and of myeloperoxidase (MPO) in the leukocytes of patients with trisomy for chromosome 21.

EXPERIMENTAL METHOD

Heparinized venous blood was used as the biological material. Gelatin in physiological saline was added in the ratio of 3:1, the mixture was allowed to stand for 30 min at 37°C, after which the leukocyte suspension was carefully drawn off. The leukocytes were washed twice with medium 199 to remove the gelatin, and lysis of the remaining red cells carried out [4]. The cell residue was resuspended in 2 ml physiological saline, distilled water was added for 30 sec, and isotonicity was restored with 2 ml of 3.5% NaCl. Complete lysis was most frequently achieved after two repetitions of this procedure. The residual leukocytes were resuspended in 3 ml of 0.1 M phosphate buffer and sonicated on the UZDN-1 ultrasonic disintegrator at 22 KHz for 1 min. The cell lysates were centrifuged at 4000 rpm on the TsLK-1 centrifuge, and G6PD and MPO activity determined in the hyaloplasm. The temperature conditions for the investigation were established by preliminary experiments. For G6PD the samples were incubated at 45°C for 10 and 20 min, and for MPO at 80°C for 10 min; samples were taken every 2 min to determine the residual enzyme activity. The results were subjected to statistical analysis by the inversion method (Wilcoxon–Mann–Whitney criterion). Protein was determined by Lowry's method [11]. G6PD activity was studied in incubation medium of the following composition (in μM): Tris-HCl, pH 7.4, 125; NADP 1, $MgCl_2$ 20, glucose-6-phosphate 2, NPO activity was determined by the method described in [9].

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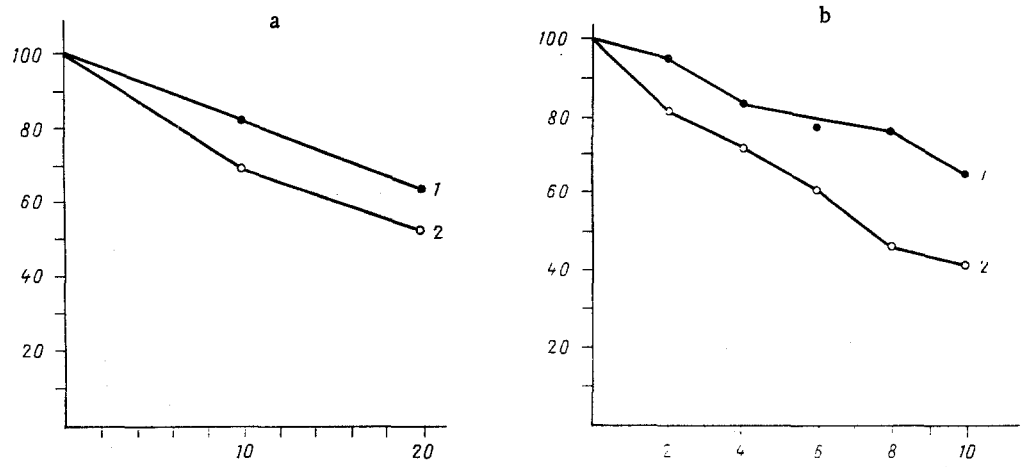

Fig. 1. Temperature sensitivity of G6PD (a) and MPO (b) in lysates of leukocytes trisomic for chromosome 21 (2) and in control (1). Abscissa, duration of exposure to temperature (in min); ordinate, percentage of residual enzymic activity after treatment. For all time intervals $P < 0.01$.

Altogether 11 patients with Down's syndrome were investigated. The diagnosis of the disease was based on the clinical picture and the results of karyologic analysis. In all cases trisomy for chromosome 21 was found. The ages of the patients ranged from 16 to 20 years.

EXPERIMENTAL RESULTS

The dynamics of changes in G6PD and MPO activity in leukocyte lysates after exposure to different temperatures is illustrated in Fig. 1. At all time intervals a statistically significant increase in temperature sensitivity of the two enzymes was discovered in the aneuploid cells compared with the control.

When the results are analyzed it is useful to refer to previous studies of the functional state of the patients' genome. Instability of the genome is known to be increased after exposure to various physicochemical factors. For instance, after x-ray irradiation chromosomal injuries arise significantly more often in cells with trisomy for chromosome 21 than in the control [2]. Similar results are obtained on incubation of peripheral blood cells with alkylating compounds [13]. Sensitivity of aneuploid cells to the action of viruses is also altered. Todaro [14] found a threefold increase in the intensity of oncogenic transformation of fibroblasts with trisomy for chromosome 21 by SV-40 virus compared with diploid cells. The frequency of chromosomal injuries in children with Down's syndrome after an attack of measles is significantly higher than in normal children after a similar attack of measles [8].

Defective repair has been demonstrated in the lymphocytes of such patients, and disturbances have been noted after injury both by x rays and by UV light [3, 10].

To sum up what has been said, it can be concluded that the genetic apparatus of these patients is characterized by instability of its function. Most probably mutation injuries take place more frequently in aberrant cells during the course of their vital activity than in normal diploid cells.

States characterized by an increased tendency toward the development of malignant diseases, by instability of the genetic apparatus, and by the more rapid development of aging are known in clinical genetics. They include the Louis–Bar syndrome, Fanconi's anemia, and progeria [6]. A study of the physicochemical properties of G6PD, 6-phosphogluconate dehydrogenase, and hypoxanthine-guanine phosphoribosyl-pyrophosphate transferase in lysates of fibroblasts obtained from patients with progeria revealed a distinct increase in temperature sensitivity of all three enzymes. In the opinion of these workers, the differences are connected with accumulation of mutant proteins in the cells of patients with progeria on account of the increased frequency of mutation injuries [7].

Increased instability of the genetic apparatus of patients with Down's syndrome, the 20-fold increase in the frequency of malignant diseases in these patients, and their defective

repair system — these are weighty evidence in support of the possible accumulation of mutant proteins in aberrant cells which, in turn, predetermines changes in the temperature sensitivity of the enzymes studied.

However, the results can be interpreted in another way. In 1969 an investigation of the temperature sensitivity of G6PD was undertaken in young and old forms of healthy human erythrocytes [5]. Because of the ease with which they can be fractionated by age composition (differential sensitivity to hypotonic solutions is usually used for this purpose), erythocytes provide one of the most convenient models for the study of the mechanisms of cell aging. These cells are known to have no translation apparatus, so that the changes discovered in the process of aging of the cell cannot be linked with any defect of function of the genetic apparatus. Increased temperature sensitivity of G6PD has been demonstrated in old erythrocytes compared with young; in the opinion of the authors concerned the difference is due to post-translation modification of this enzyme. It can accordingly be accepted that in aneuploid cells the post-translation modification of the protein molecules is disturbed. The solution to this problem would prove helpful to the explanation of the pathogenetic nature of chromosomal imbalance.

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