Changes in Blood Lymphocyte Enzyme Profile in Patients with Renal Cancer after a Course of Interferon Therapy

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Activities of NAD(P)-dependent dehydrogenases in blood lymphocytes of patients with renal cell cancer were studied. The enzyme profiles in patients were different before and after the course of immunotherapy. A relationship between changes in the enzyme status and *in vitro* cell sensitivity to IFN-α2a was detected.

Key Words: *enzymes; lymphocytes; renal cell cancer; interferon therapy*

Surgical treatment supported by adjuvant immunotherapy with IFN preparations, including recombinant IFN-α2a, is the most effective modern approach to the therapy of disseminated renal cell cancer (RCC) [1,7]. Antitumor reactivity of the organism, largely determined by functional activity of immunocompetent cells, is an important factor determining the efficiency of therapeutic measures and prediction of IFN therapy results.

It is obvious that the functional manifestations of lymphocytes, the main structural and functional element of the immune system, are determined by their metabolic reactions. Importantly that all modulators of lymphocyte functional activity primarily modify cell metabolism, switching over the substrate flow from one metabolic pathway to another, modulating cell energetics and synthetic processes; it is obvious that the metabolic events underlie disorders in the immune system. Therefore, studies of enzyme activities in immune cells from RCC patients over the course of IFN therapy is an important problem.

We studied activities of NAD(P)-dependent dehydrogenases in peripheral blood lymphocyte from RCC patients before and after the course of IFN therapy.

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MATERIALS AND METHODS

Forty-four patients with RCC (stage T3N0M0) aged 45-55 years were observed before therapy. Of these, 29 patients formed the group of observation after one course of IFN therapy (12 injections per course); 15 patients were excluded from the study in the course of treatment. Control group consisted of 35 donors of the same age without malignancies. Adjuvant immunotherapy was carried out 4 weeks after radical nephrectomy. The patients received IFN-α2a intramuscularly 3 times a week in a dose selected with consideration for individual cell sensitivity to the drug *in vitro* [5]. After one course of immunotherapy, cell sensitivity to IFN-α2a was retained in 21 of 29 patients; no cell sensitivity to the drug was detected in 8 patients.

Lymphocytes were isolated from heparin-treated venous blood by centrifugation in Ficoll-verografin density gradient [4]. Activities of glucose-6-phosphate dehydrogenase (G6PDH), glycero-3-phosphate dehydrogenase (G3PDH), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), NAD- and NADP-dependent glutamate dehydrogenases (NADGDH and NADPGDH, respectively), NAD- and NADP-dependent isocitrate dehydrogenases (NADIDH and NADPIDH, respectively), decarboxylating malate dehydrogenase (NADPMDH), and glutathione reductase (GR) were evaluated by the bioluminescent method [2]. Activities of LDH, MDH, NADGDH, and NADPGDH were evaluated by the direct and inverse reactions

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(R_LDH, R_MDH, R_NADGDH, and R_NADPGDH, respectively). Activities of the studied oxidoreductases were expressed in enzymatic units (1 U=1 μmol/min) per 10^4 cells. Enzyme preparation NAD(P):FMN oxidoreductase:luciferase from *Photobacterium leiognathi* (from Institute of Biophysics, Krasnoyarsk) was used [3]. The bioluminescence intensity was measured on a BLM 8801 bioluminometer.

The data were statistically processed using Statistica 6.0 software. The values in the compared groups were presented as the median (*Me*) and interquartile interval (LQ-UQ), where LQ is 25% and UQ 75%. The hypothesis on the reliability of the sample was verified by the Mann–Whitney test.

RESULTS

Studies of NAD(P)-dependent dehydrogenase activities in blood lymphocytes of RCC patients before IFN therapy (Table 1) showed reduced level of G6PDH

TABLE 1. Activities of NAD(P)-Dependent Dehydrogenases (μU) in Blood Lymphocytes of RCC Patients (*Me*, LQ-MQ)

Parameter	Control group (*N*=35)	RCC patients			
		before therapy (*N*=44)	after therapy (*N*=29)	IFN-α2a sensitive (*N*=21)	IFN-α2a insensitive (*N*=8)
G6PDH	3.11 (0.53-6.24)	0.46** (0.01-2.03)	1.30^{+} (0.19-3.45)	1.30 (0.83-9.85)	0.06 (0.06-2.58)
G3PDH	0.00 (0.00-1.01)	0.09 (0.00-2.50)	0.84 (0.00-1.69)	1.31** (0.11-2.59)	0.00^{o} (0.00-0.00)
LDH	11.53 (2.37-20.34)	7.85 (1.51-27.52)	6.33 (0.00-29.05)	16.50 (1.93-36.32)	0.00**^{oo} (0.00-2.50)
NADPMDH	0.33 (0.00-1.14)	0.08 (0.00-0.38)	0.24 (0.05-0.37)	0.28 (0.07-0.43)	0.07 (0.05-0.07)
NADPGDH	0.53 (0.00-4.52)	0.64 (0.00-2.48)	1.264 (0.00-6.18)	1.08 (0.00-12.90)	1.45 (0.02-4.52)
NADPIDH	2.99 (1.87-6.32)	1.96 (0.37-5.35)	1.66 (0.18-5.99)	1.62 (0.18-5.30)	7.86 (0.30-12.12)
MDH	4.58 (0.00-11.61)	32.26** (0.00-114.78)	0.00^{++} (0.00-59.18)	0.00 (0.00-59.18)	0.00 (0.00-62.67)
NADGDH	4.24 (0.00-15.48)	8.63 (0.00-28.01)	4.98^{+} (0.00-7.81)	5.18 (0.89-16.94)	1.08 (0.00-7.22)
NADIDH	0.56 (0.00-13.79)	0.21 (0.00-3.22)	7.19^{++} (0.14-13.81)	5.79 (0.16-19.20)	7.44 (0.48-10.28)
R_LDH	30.57 (0.00-52.46)	0.00** (0.00-16.51)	6.59 (0.00-37.70)	11.40 (0.00-56.61)	3.30 (0.00-10.28)
R_MDH	58.13 (24.98-114.15)	58.18 (27.51-95.54)	49.19 (25.27-67.60)	65.27 (43.40-160.48)	29.23^{o} (24.20-40.50)
GR	13.82 (7.24-36.74)	1.30** (0.00-7.63)	3.25* (0.29-11.43)	3.25 (0.30-11.69)	4.79 (0.97-14.24)
R_NADGDH	5.19 (0.00-22.70)	19.93* (5.19-32.28)	33.34* (4.49-44.92)	12.90 (4.50-44.92)	42.84 (26.05-80.19)
R_NADPGDH	25.20 (11.20-46.79)	25.27 (1.95-43.80)	13.02* (1.57-26.54)	13.02 (2.67-27.25)	14.87 (3.89-26.82)

Note. *$p<0.05$, **$p<0.01$ compared to control group; $^{+}p<0.05$, $^{++}p<0.01$ compared to the values before therapy; $^{o}p<0.05$, $^{oo}p<0.01$ compared to patients with preserved cell sensitivity to IFN-α2a.

(the key enzyme of the pentose phosphate cycle whose products are used in the macromolecular synthesis reactions [6]).

Analysis of activities of dehydrogenases with mainly energetic functions showed reduced level of R_LDH in blood lymphocytes, reflecting reduced glycolysis substrate flow. On the other hand, we observed increased activity of MDH, an enzyme determining the tricarbonic acid cycle flow intensity, which suggests intensification of aerobic intracellular processes. Intensification of the Krebs cycle substrate flow in this case is paralleled by intensification of intermediates outflow through elevation of R_NADGDH level in the amino acid metabolism reaction (Table 1). A decrease in GR in RCC patients before therapy is worthy of note (this enzyme is a component of the cellular antioxidant defense system and to a certain measure modulates lymphocyte proliferative activity) [8,9].

After a course of IFN therapy, the enzyme status of lymphocytes in RCC patients was characterized by low GR activity (Table 1). Elevation of R_NADGDH activity was paralleled by reduction of R_NADPGDH activity in comparison with the control group (Table 1).

Comparative analysis of enzyme activities in blood lymphocytes of RCC patients showed increased G6PDH and decreased MDH activities after immunotherapy in comparison with the values before therapy (Table 1). Elevation of NADIDH activity and reduction of NADGDH level in comparison with their initial values before treatment is worthy of note (Table 1).

Study of enzyme activities in blood lymphocytes of RCC patients with different cell sensitivity to IFN-α2a *in vitro* after the course of immunotherapy revealed some shifts in comparison with the control (Table 1).

Activity of G3PDH (determining transfer of lipid catabolism products to redox glycolysis reactions [10]) increased in patients with retained cell sensitivity to IFN-α2a. Activity of LDH decreased in comparison with the control in RCC patients whose cells "lost" sensitivity to IFN-α2a after immunotherapy course (Table 1).

Comparative study of enzyme activities in blood lymphocytes of patients whose cells lost sensitivity to IFN-α2a showed reduced activities of G3PDH and LDH in comparison with patients with preserved cell sensitivity to IFN-α2a, and poor activity of R_MDH, which impairs the function of the malate-aspartate shuttle (Table 1).

Hence, the results indicate differences in activities of NAD(P)-dependent dehydrogenases in RCC patients before and after IFN therapy. Production of ribose-5-phosphate and the NADPH-dependent reactions of macromolecular synthesis increased after IFN therapy. In addition, activities of enzymes metabolizing the energy-producing reactions in mitochondria were changed. Importantly that the direct LDH reaction and the level of G3PDH activity essential for the function of the glycerophosphate shuttle providing hydrogen for the Krebs cycle decreased in blood lymphocytes from RCC patients whose cells lost sensitivity to IFN-α2a; the role of the malate-aspartate shuttle in the cell energetics decreased.

These data suggest further study of the enzyme status of immunocompetent cells in RCC patients in order to optimize the adjuvant therapy of this patient population.

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