Cell Sensitivity to Reaferon in Patients with Renal Cell Cancer after Reaferon Therapy

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Individual sensitivity of blood neutrophils to IFN-α2a was studied *in vitro* in patients with renal cell cancer before and after the first course of reaferon therapy. Cell sensitivity changed after reaferon therapy in 85% patients. This necessitates evaluation of individual cell sensitivity not only before therapy, but also before the next course of therapy.

Key Words: *interferon; renal cell cancer; neutrophils; chemiluminescence*

Combination of surgery and immunotherapy is a promising approach to the treatment of patients with renal cell cancer (RCC). Data on the efficiency of IFN therapy are contradictory. Unjustified or untimely use of IFN can cause untoward effects and reduce the efficiency of immunotherapy [2-4], and therefore clinical application of IFN preparations is more effective after evaluation of individual cell sensitivity to IFN before and after each course of therapy. Neutrophils are characterized by high mobilization activity and carry IFN receptors on their surface [1,4]. Due to this, cell sensitivity to reaferon can be evaluated *in vitro* and the efficiency of therapy can be evaluated by chemiluminescent (CL) analysis.

MATERIALS AND METHODS

Dynamic observations of patients with RCC (stage $T_3N_0M_0$ according to TNM classification) were carried out at Territorial Cancer Research Center before surgical treatment (n=132), 14 days after radical nephrectomy (n=45), before reaferon therapy (n=20), and 3 weeks after it (n=20). Reaferon therapy was carried out according to the protocol developed at N. N. Blokhin Cancer Research Center (2002). Individual cell sensitivity to reaferon was evaluated *in vitro* by CL response of blood neutrophils from RCC patients without interferon and in the presence of its different doses in the reaction mixture. Chemiluminescent analysis was carried out after De Sole *et al.* The reaction mixture included 200 μl leukocyte suspension (2×10^6/ml), 20 μl donor serum, and 0.56 μM luminol. Reaferon in concentrations of 2000, 4000, and 10,000 U was added to experimental samples. The volume of the samples was brought to 510 μl with Hanks solution without phenol red. All samples were placed into a CL 3606M chemiluminometer and spontaneous CL was measured. After 45 min, 40 μl opsonized zymosan (2 mg/ml) was added to the samples and induced CL was measured. The following parameters were analyzed: time of attaining CL maximum (T_{max}), maximum intensity (I_{max}), and area under CL curve (S). Intensification of zymosan-induced CL in comparison with spontaneous CL was estimated as S_{zym}/S_{sp} and considered as activation index.

Reaferon concentration in experimental samples was estimated by the mean count of neutrophils in adult human peripheral blood, number of cells in the sample, and therapeutic doses prescribed to patients with RCC (3, 5, and 8 mln. U, respectively). Cell sensitivity to reaferon was estimated as the ratio of the neutrophil activation index of the sample with reaferon to the neutrophil activation index of the control sample. If the result was >1, the response was considered adequate and interpreted as sensitivity to the tested reaferon dose.

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The data were statistically processed using SPSS 10.0 and Statistica 6.0 software. The significance of differences in independent samplings was evaluated using Mann—Whitney test.

RESULTS

Analysis of CL of blood neutrophils from patients with RCC before surgery in response to different reaferon doses *in vitro* showed that none of the studied reaferon doses significantly modified the results of CL analysis. This cell resistance can be explained by the absence of sufficient number of appropriate receptors on the neutrophil surface before surgery or by disorders in signal transduction system of the cell in general, which, in turn, can be caused by the presence of the tumor (Table 1). *In vitro* exposure with reaferon in a concentration of 10,000 U 14 days after radical nephrectomy induced a trend to a decrease in T_{max} of spontaneous CL curve in comparison with the parameter during exposure with the dose of 4000 U in 55.55% cases. T_{max} of stimulated CL curve increased in comparison with the control in response to reaferon in a concentration of 4000 U in 64.4% cases. A trend to an increase in S_{zym} of the neutrophil CL was observed in response to reaferon in a concentration of 10,000 U in the reaction mixture in comparison with the parameter in the presence of 4000 U reaferon (Table 2). Hence, relative recovery of neutrophil sensitivity to IFN is observed in patients with RCC 14 days after nephrectomy.

Analysis of reaferon sensitivity of neutrophils *in vitro* in patients after a course of reaferon therapy revealed 4 types of the reaction of neutrophilic granulocytes to exogenous IFN-α2a. Type 1 reaction to reaferon (40% cases) consisted in a decrease in cell sensitivity to this IFN after the first course of therapy, but an increase of neutrophilic granulocyte activation index was observed in response to a higher reaferon dose than before therapy. Type 2 reaction (25% cases) was as follows: before treatment the neutrophils reacted to the medium dose (5 mln. U) by intensification of oxygen metabo-

TABLE 1. Parameters of CL of Blood Neutrophils from RCC Patients in Response to Different Reaferon Doses before Surgery (n=132; $M\pm m$)

Parameter		Control	2000 U	4000 U	10,000 U
Spontaneous CL	T_{max}, sec	707.95±47.48	808.64±59.41	909.82±71.93	949.25±72.73
	I_{max}, rel. units×10^3	53.31±3.49	52.76±3.56	50.63±3.44	50.57±3.62
	S_{sp}, rel. units×10^5	16.93±1.08	17.60±1.19	18.26±1.29	18.37±1.34
Induced CL	T_{max}, sec	780.40±28.52	735.32±24.31	768.98±25.82	796.68±27.12
	I_{max}, rel. units×10^3	172.05±11.89	165.36±10.32	193.08±11.78	190.56±11.62
	S_{zym}, rel. units×10^5	62.67±5.01	59.52±4.74	78.53±6.24	68.95±4.92
Activation index	S_{zym}/S_{sp}	4.09±0.34	4.85±0.41	6.04±0.49	5.42±0.43

TABLE 2. Blood Neutrophil CL in Patients with RCC 14 Days after Surgery in Response to Different Reaferon Doses *In Vitro* (n=45; $M\pm m$)

Parameter	Control	2000 U	4000 U	10,000 U
Spontaneous CL				
T_{max}, sec	1168.67±114.50	1101.05±112.02	1214.50±117.77	1073.59±105.50
I_{max}, rel. units×10^3	49.42±6.06	54.39±7.11	53.78±7.16	53.83±7.13
S_{sp}, rel. units×10^5	22.74±3.05	20.45±2.48	18.59±2.12	23.50±3.055
Induced CL				
T_{max}, sec	907.98±47.09	834.95±36.47	972.44±64.27*	996.56±61.37
I_{max}, rel. units×10^3	205.28±16.52	197.69±16.19	198.28±16.66	211.56±16.71
S_{zym}, rel. units×10^5	107.85±15.58	120.14±18.28	94.66±13.11	102.31±13.55$^+$
Activation index				
S_{zym}/S_{sp}	5.09±0.63	7.73±1.22	5.22±0.67	5.64±0.65

Note. *p<0.05 compared to the corresponding parameter at reaferon concentration of 2000 U; $^+$0.1>p>0.05 *vs.* the parameter at reaferon concentration of 4000 U.

lism, while after the therapeutic course they acquired the capacity to modulate the CL response in the presence of a lower reaferon dose (3 mln. U). Type 3 cell response was observed in 15% cases: cell sensitivity to reaferon dose prescribed for therapeutic course 1 did not change after treatment. Type 4 cell response (20% cases) consisted in complete disappearance of positive response of neutrophils to all the studied reaferon doses *in vitro* 3 weeks after therapeutic course 1. Hence, cell sensitivity to the studied preparation after therapeutic course 1 did not change in just 15% cases and changed in 85% patients. Reaferon dose for therapeutic course 2 was changed in 65% patients (higher dose was prescribed for 40% and lower for 25% patients). In 20% cases neutrophils lost the capacity to potentiate their compensatory abilities in the functional reaferon test, which was presumably an indication for discontinuation of immunotherapy.

Hence, in order to optimize adjuvant IFN therapy of patients with RCC it is necessary to adhere to the protocol of therapy (24-72-h intervals) determined by the mechanisms of IFN interactions with specific receptors on cell membranes and slow restoration of the number of receptors needed for binding the next portion of exogenous IFN. Individual cell sensitivity to reaferon should be evaluated before therapy and before the next course because of high probability of the need in alteration of the drug dose.

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