Lucigenin- and Luminol-Dependent Chemiluminescence of Blood Neutrophils in Patients with Renal Cancer

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High basal production of primary active oxygen forms was detected in the peripheral blood neutrophils of patients with renal cell cancer. *In vitro* stimulation of neutrophils led to more rapid release of superoxide radicals into extracellular space and to a reduction of cell capacity to more intense production of primary active oxygen forms.

Key Words: *lucigenin; luminol; chemiluminescence; neutrophils*

High incidence of renal cell cancer (RCC) responsible for 97% of all renal tumors is a pressing problem of modern oncourology. Renal cell cancer is a highly immunosensitive tumor, which prompts studies of the functions and metabolism of immune system cells in this disease [11].

Pronounced cytotoxic effect of neutrophils on tumor cells is a known fact. Rejection of some transplanted tumors is entirely determined by their infiltration by neutrophils [1,2]. The cytopathic effect of neutrophils is realized by production of reactive oxygen species (ROS) as a result of oxygen metabolism intensification, "respiratory burst", which can be evaluated by chemiluminescent (CL) analysis [7,9,14]. High correlation between neutrophil CL level and killing was proven. Differential evaluation of neutrophil production of primary and secondary ROS is carried out by CL analysis with lucigenin, which is oxidized and fluoresces under the effect of mainly superoxide anion and indirectly reflects the NADP(H) oxidase activity, and with luminol, used for evaluation of the summary activity of oxygen and other radicals (with emphasis on singlet oxygen and hydroxyl radical) [2,5,6]. ROS can simulate the effects of many hormones and neurotransmitters [12,13]. ROS generation by cells precedes other events in the intracellular information chain [10]. In addition, high interest to the "respiratory burst" is explained by possible destructive effect of ROS on cells and tissues [3,8,15].

We studied the levels of production of primary and secondary ROS by blood neutrophils in RCC patients.

MATERIALS AND METHODS

Sixty patients with locally disseminated RCC aged 40-55 years were examined before radical nephrectomy at Urology Department of Regional Oncological Center, Krasnoyarsk. Control group consisted of 56 donors.

Chemiluminescent analysis was carried out as follows. Polyglucin (1 ml) was added to 5 ml heparin-treated venous blood and the mixture was incubated for 30 min in a thermostat (37°C). Leukocytic suspension was washed twice (10 min, 400*g*) in Hanks' solution (without phenol red). The final concentration of leukocyte suspension was 2×10^6 cell/ml. The samples contained 200 μl leukocyte suspension, 20 μl donor serum (AB(IV)$Rh^{(-)}$), 240 μl Hanks' solution without phenol red, and 50 μl luminol or lucigenin (100 μg/ml; Sigma). The samples were then put into CL 3606M CL analyzer and spontaneous CL reaction was evaluated. After 45 min, 40 μl opsonized zymosan (2 mg/ml; Sigma) was added to each sample and induced CL reaction was evaluated. The results of CL analysis were evaluated by the following parameters: time of at-

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taining maximum CL intensity (Tmax), maximum CL amplitude (Imax), and area under CL curve (S). The increase in zymosan-induced CL was evaluated by the ratio of induced to spontaneous S (stimulation index).

Since CL activity is intrinsic of peripheral blood neutrophil population (other leukocytes lack this capacity or it is negligibly low), this cell fraction was not specially isolated and only neutrophil CL response is discussed in the paper [4,5,11].

The data were statistically processed using Statistica 6.0 applied software (StatSoft, Inc.). The sample was described by estimating the median (Me) and interquartile range (25 and 75 percentiles, Q_{25} and Q_{75}). The hypothesis on the statistical reliability of the studied parameters was verified using Wilcoxon's test.

RESULTS

For adequate evaluation of oxygen metabolism in neutrophils of RCC patients, we studied the parameters of lucigenin- and luminol-dependent CL of donor cells (Table 1).

Chemiluminescent analysis of functional activity of donor blood neutrophils showed that the time of attaining maximum CL was longer by 3.5 times and the area under the CL curve was by 3.6 times lower in spontaneous CL reaction with lucigenin in comparison with these parameters in samples with luminol. After induction with zymosan, the intensity and the area under the curve for lucigenin-dependent CL decreased by 5.4 and 4.8 times, respectively, in comparison with the corresponding parameters of luminol-dependent CL

TABLE 1. Luminol- and Lucigenin-Dependent CL Response of Peripheral Blood Neutrophils from RCC Patients (Me; Q_{25}-Q_{75})

Parameter	Donors (*N*=56)		Patients with RCC (*N*=60)	
	luminol-dependent reaction	lucigenin-dependent reaction	luminol-dependent reaction	lucigenin-dependent reaction
Spontaneous CL				
Tmax, sec	505.50	1989.00	1517.00	2033.00
	208.50-1502.50	1624.00-2440.00	634.00-2172.00	1334.00-2362.00
		$p<0.05$		$p<0.01$
Imax, arb. units	6.67	1.22	8.62	7.51
	3.04-18.70	0.79-8.99	3.07-20.43	4.08-23.72
S_1, arb. units	2.06	0.57	2.81	2.88
	1.23-4.60	0.36-1.93	0.99-9.54	0.70-8.33
		$p<0.001$		
Zymosan-induced CL				
Tmax, sec	1270.00	1799.00	1624.00	941.00
	862.00-1779.00	719.00-2525.00	1177.00-2121.00	215.00-1693.00
				$p<0.01$
Imax, arb. units	10.54	1.94	17.55	7.11
	4.98-41.70	1.10-6.53	5.84-48.63	4.30-21.89
		$p<0.05$		$p<0.001$
S_2, arb. units	3.78	0.78	4.48	3.30
	1.53-9.40	0.53-3.21	1.92-20.20	1.12-11.9
		$p<0.01$		$p<0.01$
Stimulation index				
S_2/S_1	1.87	1.65	1.83	1.25
	1.52-3.00	1.05-1.75	0.98-2.90	0.96-1.53
				$p<0.003$

Note. *p*: statistically significant difference from luminol-dependent CL values.

(Table 1). In the absence of tumor-associated immunosuppression, the ratio of primary to secondary ROS in donor peripheral blood neutrophils stimulated *in vitro* is 1:4. Since functional and metabolic state of donor neutrophil is not modified by any kind of pathological changes, these values were taken for normal.

Chemiluminescent analysis of functional activity of neutrophils reflecting the level of ROS production in RCC patients before intervention showed slow attaining of the maximum spontaneous lucigenin-dependent CL, similar to the time course of ROS production by donor neutrophils (Table 1). On the other hand, no appreciable differences in the maximum intensities and areas under the curve of spontaneous CL response of neutrophils were detected between the luminol and lucigenin tests, this reflecting high basal level of superoxide radical production by the cells. After induction of CL response in blood neutrophils, the duration of the reaction to the stimulus was shorter, while the maximum intensity and area under the curve of lucigenin-dependent CL decreased in comparison with luminol-dependent CL. This can indicate failure of cell membranes, because intense release of superoxide radicals from the cell is abnormal [10]. It is noteworthy that the neutrophil capacity to intensification of primary ROS production in response to *in vitro* stimulation was disordered in RCC patients, which was seen from lower stimulation index in the lucigenin test in comparison with luminol-dependent reaction. The ratio of primary to secondary ROS in peripheral blood neutrophils from RCC patients stimulated *in vitro* approached 1:1.

Hence, our results attest to pronounced intensification of spontaneous production of primary ROS with reduction of the compensatory potential of NADPH-oxidase enzyme system of the peripheral blood neutrophils and disorders in cell membrane permeability in the zymosan test in RCC patients before surgery.

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