MICROBIOLOGY AND IMMUNOLOGY

Use of Recombinant Interleukin-2 for Intrapleural Therapy of Tumor-Associated Pleurisy

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 148, No. 11, pp. 547-549, November, 2009
Original article submitted August 6, 2009

Clinical efficiency of low dose Roncoleukin was studied in 30 patients with metastatic exudative pleurisy. Intrapleural therapy proved to be highly effective (overall effect reached 84%), was well tolerated, and improved patients' quality of life.

Key Words: *metastatic pleurisy; interleukin-2; immunotherapy*

Tumor-associated (metastatic) pleurisy develops in 24-50% patients with disseminated malignant tumors and is a life-threatening complication of tumor process limiting the potentialities of systemic drug therapy and radiotherapy [1,2,7,9]. Standard treatment of patients with malignant exudation in the pleural cavity consists in fluid evacuation, systemic drug therapy, or intrapleural injection of cytostatics or sclerosing agents [1,5,7-9,11,12]. High efficiency and good tolerance of intrapleural IL-2 therapy in combination with lymphokine-activated killers (LAK cells) obtained by activation of patient lymphocytes from malignant exudation or peripheral blood with IL-2 was demonstrated [2,3,10]. However, the method of LAK cell generation is labor-consuming and expensive, this limiting its wide use [2,10]. The data on the effect of IL-2 monotherapy on the course of malignant exudation are contradictory [4,6,9].

We evaluated clinical efficiency and tolerance of intrapleural monoimmunotherapy with Roncoleukin (Biotech company) based on recombinant IL-2.

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MATERIALS AND METHODS

The study included 30 patients (5 men and 25 women aged 41-75 years) with metastatic exudative pleurisy caused by lung cancer (n=4), breast cancer (n=20), renal cancer (n=2), and ovarian cancer (n=4). The status of all patients was evaluated as medium severe. Before intrapleural immunotherapy, all patients (as a rule) received surgical treatment in combination with drug, hormone, immuno-, or radiotherapy. Before immunotherapy, 1000-3000 ml serous, serous/hemorrhagic, or hemorrhagic exudation was evacuated from the pleural cavity. Cytological analysis of pleural exudation was carried out before immunotherapy in all cases.

For immunotherapy, the pleural cavity (under local analgesia) was drained for 14 days using Pleurocan kit (B. Braun). Before injection of the drug, the cavity was maximally dried. Roncoleukin was injected into the involved pleural cavity in a dose of 1×10^6 U (1 mg) in 20 ml saline on days 1-5 and 8-12 in a total dose of 10×10^6 U. Control X-ray examination (chest X-ray in 2 projections and/or spiral computer-aided tomography of the chest organs) was carried out directly, 1 month, and then every 3 months after a course of immunotherapy.

0007-4888/09/1485-0794

TABLE 1. Immunophenotype of Pleural Exudation Lymphocyte during Intrapleural Roncoleukin Immunotherapy (*M*±*m*)

Period of study	Lymphocyte differentiation antigens							
	CD3	CD8	CD4	CD25	CD16	CD56	CD58	HLA-DR
Before immunotherapy	43.7±6.4	5.9±1.4	18.9±2.3	4.5±0.5	4.8±1.4	6.3±3.5	40.3±3.5	4.7±1.4
At the end of immunotherapy	71.1±5.2*	6.6±2.1	21.8±4.2	11.6±1.7*	12.7±2.3*	17.9±4.2*	56.5±3.4	28.8±3.7*

Note. *$p<0.05$ compared to values before immunotherapy.

Thoracoscopy with diagnostic (verification of metastatic pleurisy) and therapeutic purposes (separation of pleural adhesions and creation of an single pleural cavity) was carried out before immunotherapy in 4 patients.

Cytological studies were carried out in all patients with pleurisy before, in the middle, and at the end of intrapleural immunotherapy. Tumor exudation cells were stained with hematoxylin and eosin. Visualization and photography were carried out using an AxioVision 4 system (Carl Zeiss).

Mononuclear leukocytes (MNL) were isolated from the pleural exudation stabilized with heparin (25 U/ml) on Ficoll density gradient (ρ=1.077 g/cm^3; Sigma) by centrifugation at 400*g* for 30 min. The MNL forming an interphase ring were collected with a pipette and washed 3 times in medium 199 (PanEco). After each washing in a 10-fold volume of the medium, the cells were precipitated by centrifugation at 200*g* (10 min).

The immunophenotype of pleural exudation leukocytes was analyzed using monoclonal antibodies (Caltag Laboratories) with the corresponding antigens by flow cytofluorometry on a FACSCalibur flow cytometer (Becton Dickinson).

The data were statistically processed using Student's *t* test and WinMDI 2.8 software.

RESULTS

Before therapy, the pleural exudation usually contained an appreciable amount of tumor cells and few mature lymphocytes (2-3 per visual field). In the middle of the therapeutic course, the majority of patients had no tumor cells or these cells were in a stage of degradation, surrounded by active lymphocytes. At the end of the course, tumor cells were detected in the exudation in only 4 patients who did not respond to this therapy.

The immunophenotype of lymphocytes isolated from pleural exudation during IL-2 therapy (after 5-7 intrapleural injections) was characterized by high expression of activated antigens (CD25, HLA-DR), adhesion molecules (CD58), and natural killer antigens (CD16, CD56) in comparison with lymphocytes obtained from malignant exudation before immunotherapy (Table 1).

Intrapleural IL-2 immunotherapy was effective in 13 patients, partial effect (small volume of encapsulated fluid) was noted in 9 and stabilization (inhibi-

TABLE 2. Clinical Efficiency of Intrapleural IL-2 Immunotherapy

Clinical effect	Number of patients	
	abs.	%
Complete response	13	43
Partial response	9	30
Stabilization	4	13
No effect	4	13
Summary effect	26	86
Pleurisy relapse after therapy	7	23
Dead from disease progress	9	30
Survival more than 20 months	21	70

tion of exudate accumulation) in 4 patients (Table 2). The effect was absent in 4 cases. Summary activity of intrapleural IL-2 immunotherapy was 86%. Repeated accumulation of pleural exudation was observed in 7 patients 2 to 20 months after therapy.

Intrapleural immunotherapy was well tolerated by patients and caused virtually no side effects, except slight fever, which was easily arrested by antipyretics.

Hence, intrapleural IL-2 immunotherapy in patients with metastatic exudative pleurisy was highly effective (86%). Clinical effects of IL-2 immunotherapy were presumably due to activation of lymphocytes in the pleural cavity, which was seen from higher expression of activation antigens. A higher percentage of cells carrying natural killer markers (CD56, CD16), characterized by antitumor cytotoxic activity, was detected in the population of MNL isolated from the pleural exudate after intrapleural injection of IL-2.

Intrapleural IL-2 immunotherapy can be regarded as one of the stages of combined and/or complex therapy of patients with disseminated malignant tumors. Due to its high efficiency, good tolerance, and easy procedure, intrapleural IL-2 immunotherapy after further studies can be recommended for wide use in practical oncology for the treatment of metastatic pleurisy.

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