ONCOLOGY

Functional Activity of P-Glycoprotein in Lymphocytes of Patients with Lymphoproliferative Diseases

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Functional activity of P-glycoprotein in lymphocytes of patients with lymphoproliferative diseases was studied using rhodamine 123. Functional activity of P-glycoprotein in patients receiving a course of chemotherapy was lower than in controls. P-glycoprotein activity was higher in patients receiving more aggressive therapy. Initially activity of P-glycoprotein was higher in patients who did not respond to chemotherapy in comparison with those whose clinical status improved after a course of chemotherapy.

Key Words: *multiple drug resistance; P-glycoprotein; lymphoproliferative diseases*

The mechanisms of cell resistance to drugs are related to the functioning of ATP-dependent enzymes responsible for drug elimination from the cell. P-glycoprotein (P-gp) encoded by the MDR1 gene is a representative of this family. Some antitumor drugs (*Vinca* alkaloids, podophyllotoxins, and anthracyclines) are P-gp substrates [5].

P-gp is now extensively studied *ex vivo* in patients with hematological tumors [1,2,8,9]. P-gp activity can be measured by flow cytometery using daunorubicin, N-vinblastine and N-actinomycin D, rhodamine 123 as the substrates. Among these substrates fluorescent dye rhodamine 123 is characterized by lowest toxicity [4].

It was found that functional activity of P-gp in lymphocytes of patients with lymphoproliferative diseases depends on treatment, patient's sex, and stage of the disease [7]. We measured functional activity of P-gp in patients with lymphoproliferative diseases by the rhodamine 123 fluorescence, evaluated the relationship between P-gp activity and treatment efficiency, and studied the effects of various drugs on activity P-gp.

MATERIALS AND METHODS

Functional activity of P-gp was studied in 40 patients with lymphoproliferative diseases: 35 with non-Hodgkin's lymphomas (NHL) and 5 with chronic lympholeukemia (CLL). Eleven patients received no chemotherapy by the moment of the study (primary patients) and 29 were examined after a course of chemotherapy (treated patients). Treatment efficiency was evaluated in primary patients no earlier than 6 months after the start of chemotherapy. The patients were divided into 2 groups by the results of therapy: 1) patients with partial or long remission and 2) patients not responding to treatment (with progress of disease). Control group (13 patients) included patients of traumatological department in whom analyses were carried out before discharge from the hospital. The absence of malignant neoplasms was the main criterion for selection into control group.

Lymphocytes were isolated by centrifugation in a Ficoll-Verograffin density gradient. Functional activity of P-gp was evaluated using rhodamine 123 in the

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presence of inhibitor verapamil as described previously [3]. The concentrations of rhodamine 123 and verapamil were 5 μM and 500 μM, duration of incubation with verapamil was 1 h at 37°C.

Functional activity was evaluated on a FACS Calibur cytofluorometer (Becton Dickinson), P-gp activity was evaluated by the release of rhodamine 123 determined as the ratio of geometrical means of rhodamine 123 fluorescence in the presence and absence of verapamil.

Statistical analysis was carried out using Statistica 5 software. The significance of differences between the samplings was evaluated using the Kolmogorov—Smirnov nonparametrical test.

RESULTS

Functional activity of P-gp E_{Rh123} in primary patients (n=11) was 1.82±0.24, while in treated patients (n=29) it was significantly lower than in controls (1.57±0.14 and 1.97±0.20, respectively, p<0.05). A similar decrease in P-gp activity in lymphocytes of patients with CLL in comparison with normal lymphocytes was described previously [2]. The absence of differences in rhodamine 123 release in the control group and primary patients indicates that functional activity of P-gp is the same in tumor and normal lymphocytes.

Treated patients were divided into 3 groups: 1) 7 patients receiving only alkylating agents (cyclophosphamide and/or leukerane), 2) 7 patients receiving alkylating agents and vincristine, and 3) 15 patients receiving more aggressive therapy (alkylating agents, vincristine, and doxorubicin). Functional activity of P-gp E_{Rh123} was 1.42±0.17 in group 1, 1.17±0.09 in group 2, and 1.81±0.23 in group 3. P-gp activity was significantly higher in patients receiving doxorubicin in addition to other drugs than in patients treated with alkylating agents and vincristine (p<0.02). It is known that doxorubicin and vincristine are P-gp substrates. Their long use can modulate expression of P-gp. It was shown that doxorubicin induced expression of MDR1 mRNA in ovarian carcinoma cells [6]. The increase in P-gp expression was detected in CLL patients treated with drugs known as P-gp substrates [8,9]. Moreover, initial P-gp activity can be higher in patients requiring more aggressive therapy. However, this assumption requires special dynamic studies.

The data on P-gp activity in patients with lymphoproliferative diseases were analyzed with consideration for chemotherapy results (Table 1). In primary patients the relationship between initial P-gp activity and treatment efficiency was evaluated 6 months after the start of therapy. In this group functional activity of P-gp was higher in patients not responding to therapy in comparison with patients whose status improved after a course of chemotherapy (p<0.04). Similar analysis in the group of treated patients showed no differences between patients responding and not responding to the treatment. It should be noted that among nonresponders activity of P-gp was higher in primary patients than in treated ones (p<0.05). High activity of P-gp in some primary patients with CLL was demonstrated previously [1]. High functional activity of P-gp is probably determined by its physiological role (cytokine transport). In tumor cell P-gp can be involved in cytokine transport for autocrine mechanisms of cell growth or survival. The absence of the effect in treated patients with low P-gp activity can be explained by involvement of other mechanisms of drug resistance.

TABLE 1. Functional Activity of P-gp E_{Rh123} in Patients with Lymphoproliferative Diseases (M±m)

Treatment efficiency	Primary patients	Treated patients
Effective	1.57±0.22*	1.57±0.21
	(7)	(17)
Ineffective	2.44±0.50+	1.55±0.11
	(4)	(12)

Note. Number of patients is shown in parentheses. *p<0.04 compared to primary patients resistant to therapy; +p<0.05 compared to treated patients resistant to therapy.

Hence, our data indicate that functional activity of P-gp in treated patients is below the control and is higher in patients receiving more aggressive therapy. In some of primary patients initially high P-gp activity is responsible for inefficiency of further therapy.

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