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Use of Glutathione Inhibitors and Glutathione Transferases to Overcome Tumor Resistance to Cytostatics *In Vivo*

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Preincubation of cells of BDF1 hybrid mice with P388 leukemia with doxorubicin and buthionine sulfoximine leads to the manifestation of a therapeutic effect of the antibiotic. Injection of buthionine sulfoximine and ethacrinic acid to mice with leukemia does not alter the therapeutic effect of the antibiotic.

Key Words: *glutathione; glutathione transferases; buthionine sulfoximine; ethacrinic acid; resistance*

The possible contribution of glutathione and glutathione-dependent enzymes to the maintenance of tumor cell resistance to cytostatics is being widely discussed at present. The levels of glutathione and glutathione-S-transferases have been shown to be increased in cells with multiple drug resistance phenotypes and in those resistant to alkylating agents [5,7]. In this connection the use of drugs inhibiting the activity of these enzymes and/or reducing the glutathione content is one of the methods of overcoming tumor cell resistance to cytostatics. Some reports advocate such an approach. However, these studies were carried out *in vitro*, and, hence, the possibility of using inhibitors of glutathione and glutathione transferases to overcome tumor resistance to cytostatics *in vivo* is still undocumented. The importance of such studies is self-evident, for obviously it is sometimes virtually impossible to attain *in vivo* the drug concentrations which are used *in vitro*.

We investigated the effects of buthionine sulfoximine (BSO), a glutathione-S-transferase inhibitor, and ethacrinic acid (EA), a glutathione inhibitor, on the abolishment of doxorubicin (DX) resistance of mouse leukemia P388 with induced antibiotic resistance (P388/DX). Previously we demonstrated a 3.5-fold increase of glutathione transferase activity in P388/DX leukemia cells in comparison with the initial sensitive strain, this permitting us to use this tumor strain as a model [1].

MATERIALS AND METHODS

Experiments were carried out with hybrid BDF1 mice aged 2 to 3 months. P388 leukemia cells sensitive to DX (P388/0, tumor strain bank of the Cancer Research Center, Russian Academy of

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Medical Sciences) and cells with induced antibiotic resistance (P388/DX) were intraperitoneally transplanted in a dose of 1×10^6 cells in 0.2 ml of medium 199. Officinal DX in a dose of 5 mg/kg body weight was intraperitoneally injected 24 h after tumor transplantation.

BSO in a dose of 1 g/kg was injected once 20 min before the cytostatic. EA in a dose of 40 mg/kg was injected twice 20 min before and 2 h after DX injection.

Leukemia P388/0 and P388/DX cells with DX and BSO were incubated in medium 199 at 37°C for 2 h before being transplanted to animals. The DX concentration was 0.8 mg/ml and the BSO concentration 0.5 mg/ml.

The therapeutic effect was assessed by animal survival. The modifier was considered effective if longevity was at least 25% prolonged [2]. Each group consisted of 10 animals.

Glutathione transferase activity in the operation material was measured routinely [4]. A 1 mM solution of 1-chloro-2,4-dinitrobenzene was used as substrate. At least 3 measurements were carried out in each sample. Protein concentration was measured after Bradford [3]. Results were statistically processed after Fisher-Student.

RESULTS

In order to prolong the exposure to the modifier, in preliminary experiments P388/DX leukemia cells were incubated with BSO for 2 h before being transplanted to animals (Table 1). The modifier was used in doses equal to those used *in vitro* [5]. The influence of BSO on the therapeutic effect of DX in mice with P388 leukemia could not be evaluated in these experiments because of the high therapeutic efficacy of the cytostatic (groups 3 and 4). Preincubation of P388/DX leukemia cells with BSO led to the manifestation of the

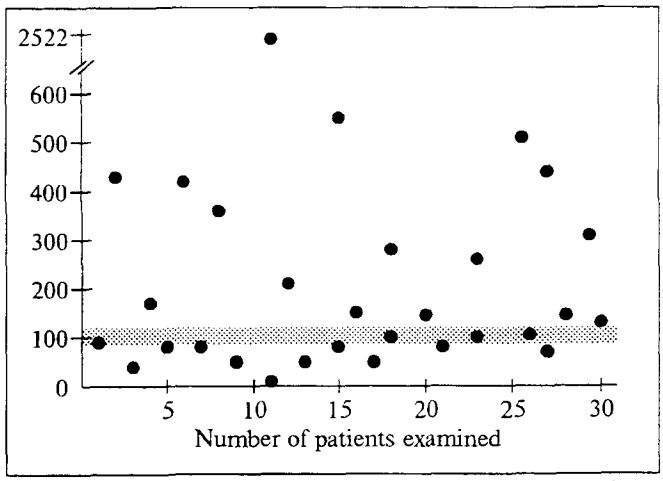

Fig. 1. Level of glutathione transferase activity in patients with different forms of non-small-cell pulmonary carcinoma. *Ordinate*: glutathione transferase activity in tumor cells in percent *vs.* the control (glutathione transferase activity in normal lung tissue). Portion marked: fluctuations in the level of glutathione transferase activity in normal lung tissue.

therapeutic effect of the antibiotic. The life span was prolonged by 31% in this group of animals, *vs.* 6% in groups 7 and 8 without the modifier, in comparison with control group 5.

Injection of BSO to animals with leukemia P388/0 and P388/DX did not alter the therapeutic action of DX (Table 2). The survival period was prolonged by 164 and 165%, respectively (groups 3 and 4), in animals administered the antibiotic alone or in combination with the modifier, in comparison with the control (group 1). In similar groups of animals with leukemia P388/DX the life span was prolonged by 6 and 9%, respectively (groups 7 and 8) *vs.* the control (group 5).

Table 3 presents the results of a study of the effect of EA on the abolishment of P388/DX leukemia resistance to DX. EA is known to lower the glutathione level and to reduce tumor cell resistance to cytostatics *in vitro* [6]. However, *in vivo* EA did not induce manifestation of the therapeutic effect

TABLE 1. Influence of BSO on the Therapeutic Effect of DX in Mice with P388 Leukemia Sensitive to the Antibiotic and with Induced Resistance to it after Preincubation of Cells *in Vitro* ($M \pm m$)

Group №	Experimental conditions	Modifier	Mean survival period, days	Prolongation of survival period, % of control
1	P388/0		13.7±0.1	
2	P388/0	BSO	13.4±0.2	−2
3	P388/0+DX		>90	
4	P388/0+DX	BSO	>90	
5	P388/DX		14.9±0.5	
6	P388/DX	BSO	14.8±0.5	−1
7	P388/DX+DX		15.8±0.5	6
8	P388/DX+DX	BSO	19.6±1.0	31

TABLE 2. Influence of BSO on the Therapeutic Effect of DX in Mice with P388 Leukemia Sensitive to and with Induced Resistance to the Antibiotic ($M \pm m$)

Group №	Experimental conditions	Modifier	Mean survival period, days	Prolongation of survival period, % of control
1	P388/0		13.1±0.2	
2	P388/0	BSO	13.4±0.3	2
3	P388/0+DX		34.6±2.0	164
4	P388/0+DX	BSO	34.7±4.1	165
5	P388/DX		16.5±1.1	
6	P388/DX	BSO	16.6±0.6	1
7	P388/DX+DX		17.5±0.9	6
8	P388/DX+DX	BSO	18.0±1.3	9

TABLE 3. Influence of EA on the Therapeutic Effect of DX in Mice with P388 Leukemia Sensitive to and with Induced Resistance to the Antibiotic

Group №	Experimental conditions	Modifier	Mean survival period, days	Prolongation of survival period, % of control
1	P388/0		11.6±0.2	
2	P388/0	EA	13.4±0.3	15
3	P388/0+DX		26.1±1.9	125
4	P388/0+DX	EA	20.9±1.0	80
5	P388/DX		11.8±0.3	
6	P388/DX	EA	13.5±0.2	14
7	P388/DX+DX		11.9±0.2	0
8	P388/DX+DX	EA	12.9±0.1	8

of DX in P388/DX leukemia cells (groups 7 and 8). EA administration reliably reduced the therapeutic effect of DX on P388/0 leukemia cells. In animals with P388/0 leukemia treated with DX alone survival prolongation was 125%, as against 80% in animals treated with the antibiotic and modifier (groups 3 and 4), *vs.* the control (group 1).

Hence, none of the tested resistance modifiers can be said to influence the therapeutic effect of DX *in vivo*. Nonetheless, we consider that the search for modifiers of tumor cell resistance to cytostatics should be continued among the agents inhibiting glutathione transferase activity, because an increase of the activity of this enzymatic system, as mentioned above, has been demonstrated for a number of tumors with the multiple drug resistance phenotype and for tumors resistant to alkylating agents [5,7]. Our study revealed an increased activity of glutathione transferases in 17 out of 30 (57%) patients with different histologic variants of non-small-cell pulmonary carcinoma little sensitive to chemotherapy (Fig. 1).

We consider that the failure of BSO and EA to abolish tumor resistance to DX *in vivo* may be due to insufficiently long exposure of tumor cells to the modifier under such conditions. Moreover, we may assume that BSO and EA change the pharmacokinetics of DX and/or are characterized by a different pharmacologic activity. Evidently, glutathione and glutathione transferase inhibitors proposed to abolish tumor resistance to cytostatics should meet the following criteria: they should be specific inhibitors of this enzymatic system, have low toxicity, and possess no other pharmacologic activity.

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