Use of Multiple Drug Resistance Modifiers to Overcome Tumor Resistance to Cytostatics in an *In Vivo* System

S. M. Sitdikova, F. V. Donenko, A. O. Kabieva, B. E. Polotskii, and L. V. Moroz

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Quinine and halidor, modifiers of multiple drug resistance *in vitro*, did not abolish tumor resistance to doxorubicin *in vivo*, as was shown in male hybrid BDF_1 mice with transplanted leukemia P 388 sensitive to doxorubicin and with induced resistance to it. Induced hyperglycemia did not lead to the manifestation of a therapeutic effect of doxorubicin in mice with leukemia P 388 sensitive to the antibiotic and with induced resistance to it.

Key Words: *multiple drug resistance; quinine; halidor; induced hyperglycemia*

Study of the possible ways of overcoming tumor resistance to cytostatics is a problem in chemotherapy of malignant tumors. There is an ongoing search for substances capable of eliminating multiple drug resistance (MDR) — a form of tumor resistance to cytostatics. The basic criteria for the primary selection of MDR modifiers is the presence of at least two polyunsaturated aromatic rings and a tertiary nitrogen atom in the molecule [5]. Systems of mathematical simulation of the modifying properties of studied substances and a panel of cell lines for *in vitro* screening thereof have been developed and described [7]. One way of controlling MDR *in vitro* is to add compounds blocking the function of P-glycoprotein — a protein whose expression has been observed on tumor cell membranes with the MDR phenotype — to the incubation medium; in addition, compounds are used, affecting other specific mechanisms of MDR [1]. On the other hand, it is obvious that their *in vivo* use may be either ineffective or impossible because of high toxicity. Despite the importance of such investigations, there are virtually no reports about the effects of MDR modifiers on tumor cell resistance to cytostatics *in vivo*. Hence, the purpose of this investigation was to study the possibility of using *in vivo* the MDR modifiers of different pharmacological groups capable of eliminating the resistance of tumor cells to cytostatics *in vitro*. Mouse leukemia P 388 with induced resistance to doxorubicin (DR) was used as a model.

Department for the Study of Antitumor Drugs, Department of Thoracal Oncology, N. N. Blokhin Cancer Research Center, Russian Academy of Medical Sciences, Moscow (Presented by Yu. N. Solov'ev, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

Experiments were carried out with male hybrid BDF_1 mice aged 2 to 3 months. Leukemia P 388 cells sensitive to DR (P 388/0, bank of tumor strains, Cancer Research Center, Russian Academy of Medical Sciences) and with induced resistance to the antibiotic (P 388/DR) were transplanted intraperitoneally or intramuscularly in a dose of 1×10^6 cells in 0.2 ml medium 199. DR (officinal drug) was injected intraperitoneally in a dose of 5 mg/kg b.w. 24 h after tumor transplantation. The studied modifiers quinine (officinal agent) and halidor (officinal agent) were injected twice in doses of 40 mg/kg 20 min before DR and 120 min after it.

The level of tumor cell resistance was assessed from the incorporation of ^{3}H-uridine after short-term incubation [6]. A tumor cell suspension (500,000/ml)

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was incubated with DR in different concentrations for 3 h at 37°C. ^{3}H-Uridine was added 2 h after the beginning of incubation. Aliquots of cell suspensions were transferred onto cellulose filters, the acid-soluble fraction was washed, and the incorporated label scintillated. The concentration of DR inhibiting label incorporation by 50% (IC_{50}) was estimated graphically.

In order to induce artificial hyperglycemia (HG), the animals were injected 10 g/kg 40% glucose solution 20 min before DR.

The therapeutic effect was assessed from the life span of animals. The modifier was considered effective if the life span was prolonged at least 25% [2]. Each group consisted of 10 mice.

RESULTS

Table 1 presents the effect of quinine on the cytotoxic action of DR in mice with leukemia P 388 sensitive to the antibiotic and with induced resistance to it. Previous studies showed that injection of quinine in the dose used blocks the release of cytostatics from resistant cells [3]. However, *in vivo* experiments demonstrated that quinine lessens the thera-

TABLE 1. Effect of Quinine on the Therapeutic Action of Doxorubicin (DR) in Mice with P 388 Leukemia Sensitive to the Antibiotic and with Induced Resistance to It (*M±m*)

Group No.	Group	Modifier	Mean life span, days	Prolongation of life span, % of control
1	P 388/0	-	13.1±0.1	
2	P 388/0	Quinine	12.1±0.4	0
3	P 388/0+DR	-	23.1±0.7	76
4	P 388/0+DR	Quinine	19.5±1.2	49
5	P 388/DR	-	13.1±0.1	
6	P 388/DR	Quinine	13.2±0.2	0
7	P 388/DR+DR	-	13.2±0.1	0
8	P 388/DR+DR	Quinine	13.6±0.2	4

TABLE 2. Effect of Halidor on the Therapeutic Action of Doxorubicin (DR) in Mice with P 388 Leukemia Sensitive to the Antibiotic and with Induced Resistance to It (*M±m*)

Group No.	Group	Modifier	Mean life span, days	Prolongation of life span, % of control
1	P 388/0	-	13.1±0.2	
2	P 388/0	Halidor	13.3±0.4	2
3	P 388/0+DR	-	25.7±0.5	96
4	P 388/0+DR	Halidor	22.5±1.2	71
5	P 388/DR	-	13.2±0.5	
6	P 388/DR	Halidor	13.3±0.3	1
7	P 388/DR+DR	-	13.8±0.4	5
8	P 388/DR+DR	Halidor	13.7±0.3	4

TABLE 3. Effect of Induced Hyperglycemia (HG) on the Therapeutic Action of Doxorubicin (DR) in Mice with P 388 Leukemia Sensitive to the Antibiotic and with Induced Resistance to It (*M±m*)

Group No.	Group	Modifier	Mean life span, days	Prolongation of life span, % of control
1	P 388/0	-	22.1±0.1	
2	P 388/0	HG	22.1±0.4	0
3	P 388/0+DR	-	20.8±0.9	0
4	P 388/0+DR	HG	19.5±1.2	0
5	P 388/DR	-	20.2±0.6	
6	P 388/DR	HG	21.2±0.8	5
7	P 388/DR+DR	-	19.2±1.1	0
8	P 388/DR+DR	HG	22.2±0.8	10

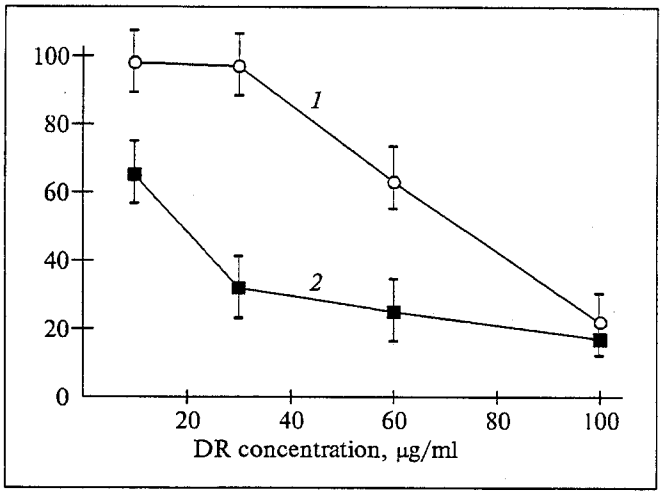

Fig. 1. Effect of halidor on doxorubicin (DR) cytotoxicity in P 388 leukemia cells with induced resistance to the antibiotic. Ordinate: ^3H – uridine incorporation in P 388/DR leukemia cells (in % of the control). *1*) P 388/DR leukemia cells; *2*) leukemia cells P 388/DR + halidor in a concentration of 8 μg/ml.

peutic effect of DR in mice with P 388/0 leukemia. For animals with P 388/0 leukemia administered the antibiotic alone and the antibiotic with the modifier the life span was prolonged 76 and 49%, respectively (groups 3 and 4). Injection of quinine did not alter the mean survival of animals with P 388/DR leukemia treated with DR (groups 7 and 8).

Similar results were observed as regards the effect of halidor on the therapeutic effect of DR in mice with leukemia P 388/0 and P 388/DR (Table 2). The life span was prolonged 96 and 71% in the groups with P 388/0 leukemia treated with the antibiotic alone and antibiotic+modifier, respectively (groups 3 and 4). For similar groups of animals with P 388/DR leukemia the life span was prolonged only 5 and 4%, respectively (groups 7 and 8). At the same time, the results of *in vitro* studies led us to expect a higher efficacy of halidor as an MDR modifier. Study of its effect on the cytotoxicity of DR for leukemia P 388/DR cells showed a more than 12-fold reduction of cell resistance to the cytostatic (Fig. 1).

Previous experiments demonstrated an increased release of cytostatics from P 388/DR leukemia cells, which suggested the contribution of ATP-dependent transport to the cell resistance to antitumor agents and an increased acidification of the tumor consisting of resistant cells [4]. Table 3 presents the effects of induced HG on the therapeutic effect of DR in mice with leukemia P 388/0 and P 388/DR. HG in the regimen used lowered the pH of the cells. However, it has been shown *in vivo* that HG does not lead to the manifestation of a therapeutic effect of DR in mice with leukemia P 388/0 and P 388/DR. It is noteworthy that in these experiments leukemia P 388 was transplanted to mice intramuscularly in a dose of $1 \cdot 10^6$ cells in order to induce acidosis.

Hence, the studied agents are not potent modifiers of MDR *in vivo*. Probably, the scarcity of reports about *in vivo* testing of MDR modifiers is due to their low efficacy in comparison with that *in vitro*.

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