The reported data are preliminary results of ongoing research into the mechanisms of the mutagenic effects of long-term psychoemotional stress connected with the participation of immunocompetent cells, as well as an analysis of the possibilities of suppressing mutagenic effects by immunocorrection.

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ONCOLOGY

Cross Resistance to Cytostatics of P388 Leukemia Cells with Induced Resistance to Doxorubicin

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

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Studies of the mechanisms by which tumor cells develop resistance to cytostatics and investigation of possible ways of overcoming this resistance are of key importance for the chemotherapy of malignant neoplasms. One of the commonest and best studied forms of resistance is multiple drug resistance (MDR), which is characterized by the ability of tumor cells to withstand the effects of a broad class of antineoplastic drugs of natural origin and their synthetic analogs [1]. A remarkable feature of MDR is that treatment of tumor cells with only one cytostatic is in most instances sufficient for these cells to become resistant to a range of cytostatics [1]. Accordingly, if an effective strategy for the chemotherapy of tumors with an MDR phenotype is to be developed, their cross resistance and collateral sensitivity to cytostatics of various groups must be known. The purpose of this study was to examine the cross resistance to several cytostatics of murine P388 leukemia cells with induced resistance to doxorubicin. This cell sub-

Department for the Study of New Antineoplastic Drugs, Cancer Research Center, Russian Academy of Medical Sciences, Moscow. (Presented by N. N. Trapeznikov, Member of the Russian Academy of Medical Sciences)

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TABLE 1. Effect of Cytostatics of Natural Origin on the Mean Life-Span of Mice With Leukemia P388 and Induced Resistance to Doxorubicin (DX). The Values are Means±SEM

Group	Cytostatic, mg/kg	Mean life-span, days	Increase in life-span, %*
P388/0	–	10.1±0.1	
P388/0	DX (5.0)	18.5±0.7	83
P388/0	–	10.0±0.0	
P388/DX	DX (5.0)	10.1±0.1	1
P388/0	–	13.4±0.9	
P388/0	DN (5.0)	19.8±0.6	48
P388/DX	–	13.1±0.3	
P388/DX	DN (5.0)	13.3±0.2	2
P388/0	–	13.4±0.9	
P388/0	CR (2.0)	16.7±0.8	25
P388/DX	–	13.1±0.3	
P388/DX	CR (2.0)	13.1±0.6	0
P388/0	–	12.0±0.0	
P388/0	ACD (0.3)	15.9±0.3	33
P388/DX	–	10.6±0.4	
P388/DX	ACD (0.3)	10.9±0.4	3
P388/0	–	12.0±0.0	
P388/0	VC (1.0)	18.4±0.6	53
P388/DX	–	10.6±0.4	
P388/DX	VC (1.0)	10.0±0.5	0
P388/0	–	13.1±0.9	
P388/0	MTC (2.0)	24.2±2.4	85
P388/DX	–	13.1±0.1	
P388/DX	MTC (2.0)	14.4±0.2	10
P388/0	–	11.8±0.2	
P388/0	BL (100.0)	18.2±0.8	54
P388/0	BLM (30.0)	19.8±0.3	68
P388/DX	–	10.8±0.4	
P388/DX	BL (100.0)	19.6±0.4	81
P388/DX	BLM (30.0)	20.4±0.5	89

Note. * Relative to untreated control mice. DN – daunomycin; CR – carminomycin; ACD = actinomycin D; VC = vincristine; MTC – mitomycin C; BL – bleomycin; BLM – bleomycetin.

line had been obtained and described by us previously [5].

MATERIALS AND METHODS

For the study, male BDF1 (C57Bl/6×DBA) hybrid mice aged 2-3 months were used. P388 leukemia cells with induced resistance to doxorubicin (P388/DX cells) were obtained through selection from P388 leukemia cells (P388/O cells - the original stain received from the bank of tumor strains maintained at the Cancer Research Center, Moscow) in the course of treating mice with this antibiotic in low doses. A total of 35 passages were required for DX resistance to develop.

Tumor cells were transplanted into mice intraperitoneally, 1 to 10 cells in 0.2 ml of medium 199 per mouse. Antineoplastic drugs - doxorubicin (DX), daunomycin (DN), carminomycin (CR), actinomycin D (ACD), vincristine (VC), mitomycin C (MTC), bleomycin (BL), bleomycetin (BLM), cyclophosphane (CPH), and 5-fluorouracil (5-FU) - were also injected intraperitoneally, in doses indicated in the tables, 24 h after tumor cell transplantation.

The therapeutic efficacy of the cytostatics was assessed by noting how long the mice survived. A cytostatic was considered to be effective if survival was prolonged by at least 25% [3]. There were 10 mice in each group.

Cells of the cell lines used (S180 sarcoma, L1210 leukemia, and P388 leukemia) with or without induced DX resistance were cultured in RPMI 1640 medium supplemented with 10% bovine serum and 1% L-glutamine, in a CO incubator at 37°C. DX resistance was maintained by adding DX after each change of the incubation medium. To this, H-uridine was added for 1 hour. After incubation, aliquots of the cell suspension to be tested were applied to a filter, the acid-soluble fraction was extracted, and the incorporated label was measured in a scintillation counter [4]. The results were treated statistically using Fisher-Student's test. The differences were considered significant at $p<0.05$.

RESULTS

For this study, we selected drugs that are most commonly used clinically in the treatment of cancer patients and vary in structure and mechanism of action. As shown in Table 1, DX, DN, CR, ACD, VC, and MTC administered in doses that prolonged by 25% to 83% the survival of mice

TABLE 2. Effect of Cyclophosphane (CPH) and 5-Fluorouracil (5-FU) on the Life-Span of Mice With Leukemia P388 and Induced Resistance to Doxorubicin (DX). The Values are Means±SEM

Group	Cytostatic, mg/kg	Mean life-span, days	Increase in life-span, %*
P388/0	–	10.1±0.2	
P388/0	CPH (50.0)	15.9±0.3	57
P388/0	CPH (100.0)	17.4±1.2	72
P388/DX	–	10.8±0.4	
P388/DX	CPH (50.0)	14.9±0.4	38
P388/DX	CPH (100.0)	17.9±0.8	66
P388/0	–	12.0±0.0	
P388/0	5-FU (100.0)	21.1±1.8	76
P388/DX	–	10.6±0.4	
P388/DX	5-FU (100.0)	14.1±1.3	33

Note. * Relative to untreated control mice.

TABLE 3. Levels of H−Uridine Incorporation into Cells of Mice with Leukemia P388, L1210, or Sarcoma S180 and With or Without Induced Resistance to Doxorubicin (DX). The Values are Means±SEM

Group	H-Uridine incorporation, cpm		p
	Without resistance	With resistance	
P388	12 912.0±1082.0	8068.0±1506.0	<0.05*
L1210	13 214.5±268.5	8197.0±15.0	<0.01*
S180	11 119.0±439.5	9179.3±200.7	<0.01*

Note. * Significant difference from the level of H−uridine incorporation in mice without induced resistance to DX.

with P388/O leukemia failed to do so in those with P388/DX leukemia which survived for the same mean period as the untreated mice. BL and BLM prolonged by >80% the mean survival of P388/DX leukemia mice and by 54% and 68%, respectively, that of P388/O leukemia mice in the same doses (Table 1, group 7).

The results presented in Table 1 indicate that the P388/DX leukemia cells with resistance induced solely to DX are also resistant to a majority of the other cytostatics of natural origin. Of the tested drugs from this group, collateral sensitivity was retained by P388/DX leukemia cells only to BL and BLM. Although these two antineoplastic antibiotics are water-soluble polypeptides, they are alkylators in their mechanism of action. It is therefore highly probable that the failure of P388/DX leukemia cells to exhibit cross sensitivity to these drugs is due to the physicochemical properties of their molecules.

The survival of mice with P388/O or P388/DX leukemia was also prolonged by CPH and 5-FU (Table 2). It should be noted that the therapeutic efficacy of these two drugs in P388/DX leukemia mice was somewhat lower than in the original sensitive strain, which can be attributed to decreased permeability of low-molecular compounds, such as CPH and 5-FU, into cells with the MDR phenotype. For example, as we found earlier, less amino acids were taken up by MDR cells than by those sensitive to cytostatics [2]. Similar results were obtained when measuring the level of H-uridine incorporation into P388 leukemia, L1210 leukemia, and S180 sarcoma cells, both those sensitive to DX and those with induced resistance to this drug (Table 3). Thus, the resistant cells were found to have incorporated significantly less of the labeled nucleotide than did their sensitive counterparts.

In conclusion, this study has shown that P388 leukemia cells with induced resistance to doxorubicin display cross sensitivity to daunomycin, carminomycin, actinomycin D, vincristine, and mitomycin and retain their collateral sensitivity to bleomycin, bleomycetin, cyclophosphane, and 5-fluorouracil.

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