Combined Effect of bis-β-Chloroethylamine Estrogen Derivatives and Doxorubicin on Proliferation of Sensitive and Resistant MCF-7 Cells

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The sensitivity of normal (MCF-7/WT) and doxorubicin-resistant (MCF-7/R) breast cancer cells to the antiproliferative effect of ethynylestradiol 11α-derivatives with the cytostatic residue in the 3-position of the steroid ring (antiestrogen cytostatics) was studied by evaluating cell viability using methylthiazole tetrazolium staining. The antiproliferative effects of these agents on cell lines in the presence of doxorubicin were compared. Antiestrogen cytostatics produced weaker cytostatic effect on MCF-7/WT cells, but more potent cytostatic effect on MCF-7/WT cells compared to those of doxorubicin. Moreover, administration of these agents in combination with doxorubicin more significantly suppressed proliferation of tumor cells. Accumulation and efflux of cytostatic doxorubicin in MCF-7/R cells were studied in the presence and absence of antiestrogen cytostatic Po716. Confocal laser microscopy showed that doxorubicin accumulation in MCF-7/R cells in the absence of Po716 took 20 min, while in the presence of antiestrogen cytostatic this process took 5 min. The rate of doxorubicin transport from tumor cells was much lower in the presence of the test antiestrogen cytostatic. Our results suggest that antiestrogen cytostatics increase the sensitivity of resistant MCF-7/R cells to doxorubicin by modulating the mechanisms of multidrug resistance of tumor cells.

Key Words: *antiestrogen cytostatics; multidrug resistance*

The search for compounds reducing multidrug resistance (MDR) of tumor cells to cytostatic drugs is an urgent problem of modern chemotherapy. The development of MDR in tumor cells is an important problem of oncology, because repeated courses of chemotherapy induce resistance of tumor cells to therapeutic drugs [2,5,7].

One of the approaches to prevent MDR is associated with pharmacological correction of this disorder with various groups of drugs.

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Attention was paid to C ring-transformed estrogens with bis-β-chloroethylamine-containing fragment. Their cytostatic activity was demonstrated on some cultured tumor cells (*e.g.*, CaOV and L-929) and transplantable tumor (rat breast carcinoma) [3,4]. These compounds also exhibit antiestrogen activity *in vivo* [9]. These compounds were chosen because they are characterized by membranotropic activity [1] and, hence, can modulate the MDR system. The formula and position of radicals are shown in Fig. 1 and Table 1. 11α-Derivatives of ethynylestradiol with the β-chloroethylamine residue in the 3-position are shown in Table 1.

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TABLE 1. Radicals in bis-β-Chloroethylamine Derivatives of Transformed Estrogens

Estrogen cytostatic	R_1	R_2	R_3	R_4
Po715 (11α)	HCOO	CH_3COO	C=CH	CytO*
Po716 (11α)	CH_3COO	CH_3COO	C=CH	CytO*

Note. *CytoO, COXN $(CH_2CH_2CL)_2$; X=$CH_2C_6H_4$.

MATERIALS AND METHODS

Experiments were performed on monolayer culture of MCF-7/WT and MCF-7/R HV cells of human breast cancer. The cells were cultured in standard DMEM (Sigma) containing 10% heat-inactivated fetal bovine serum (Bioclot) and gentamicin (40 μg/ml, Ferane).

For evaluation of cytostatic activity, the cells were incubated with antiestrogen cytostatics, doxorubicin, or their combinations in final concentrations of 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} M for 3 days. Samples without the test substances served as the control. Cytostatic activity of the test compounds was determined in the MTT test. The number of viable cells in samples incubated in the presence or absence of the test compounds was estimated in the MTT test.

The accumulation and efflux of cytostatic doxorubicin in normal and tumor cells were studied by confocal laser microscopy using a LSM-5 Pascal microscope [6,11]. MCF-7/R tumor cells served as the object of the study. Doxorubicin and antiestrogen cytostatics were added to the incubation medium to a concentration of 10^{-6} M.

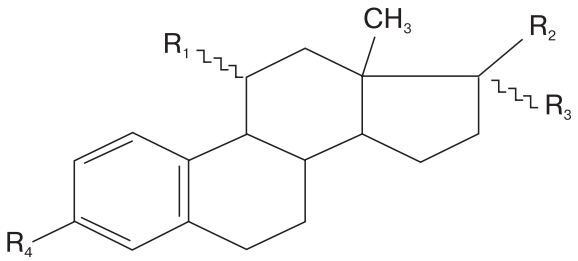

Fig. 1. Scheme of radicals in bis-β-chloroethylamine derivatives of transformed estrogens.

RESULTS

We studied the cytostatic effect of antiestrogen cytostatics, antitumor antibiotic doxorubicin, and their combination on MCF-7/WT and MCF-7/R tumor cells (Tables 2 and 3).

Doxorubicin exhibited the most potent cytostatic activity against MCF-7/WT cells; in the maxi-

TABLE 2. Effect of Individual and Combined Treatment with Po715, Po716, and Doxorubicin on Viability of Doxorubicin-Sensitive Breast Cancer MCF-7/WT Cells (%, *M*±*m*)

Concentration	Po715	Po716	Doxorubicin	Doxorubicin in combination with	
				Po715	Po716
10^{-4}	30.2±1.2*	45.1±0.2*	70.3±5.6*	69.5±2.1*	55.0±2.3*
10^{-5}	29.5±0.5*	38.3±1.5*	65.5±7.8*	68.3±0.7*	67.5±2.2*
10^{-6}	20.0±1.1*	38.3±1.1*	60.1±2.3*	60.5±4.5*	61.0±2.3*
10^{-7}	21.9±0.9*	35.1±4.5*	55.2±1.1*	58.5±1.2*	55.5±2.1*

Note. Here and in Table 3: *$p \leq 0.005$ compared to the control. Control, 0% decrease in cell viability in the absence of preparations.

TABLE 3. Effect of Individual and Combined Treatment with Po715, Po716, and Doxorubicin on Viability of Doxorubicin-Resistant Breast Cancer Cells (%, *M*±*m*)

Concentration, M	Po715	Po716	Doxorubicin	Doxorubicin in combination with	
				Po715	Po716
10^{-4}	48.1±2.1*	77.1±3.5*	30.1±3.2*	87.2±4.1*	88.1±2.7*
10^{-5}	46.0±1.4*	76.3±3.3*	25.2±1.2*	87.0±2.2*	87.1±4.4*
10^{-6}	46.4±1.0*	65.1±2.1*	24.1±1.1*	86.1±4.7*	84.1±5.6*
10^{-7}	45.1±2.0*	66.2±2.2*	23.0±1.4*	86.2±4.5*	82.1±7.1*

mum concentration it decreased cell viability by 70.3%. Antiestrogen cytostatics decreased tumor cell viability by 30.2 and 45.1%. Combined treatment with these compounds had an intermediate effect on cell viability.

Doxorubicin had little effect on cytostatic-resistant MCF-7/R cells and in the maximum concentration decreased cell viability by 30.1%. Antiestrogen cytostatics exhibited higher cytostatic activity (48.1 and 77.1%). Combined treatment with antiestrogen cytostatics and doxorubicin most significantly decreased tumor cell viability (by 87.2 and 88.1% in a concentration of 10^{-4} M).

Published data show that MDR is associated with increased activity of P-glycoprotein, while the methods for MDR prevention are usually based on modulation of P-glycoprotein activity [2,5,7].

It was hypothesized that potentiation of the cytostatic effect of doxorubicin on doxorubicin-resistant MCF-7/R cells in the presence of antiestrogen cytostatic results from its effect on P-glycoprotein, because antiestrogens significantly decrease MDR [8].

For verification of this hypothesis, accumulation and efflux of cytostatic doxorubicin in tumor cells before and after incubation with estrogen cytostatic were evaluated by fluorescence of the intracellular space. Doxorubicin-resistant MCF-7/R cells accumulated doxorubicin for 30 min after its addition to the well (mean fluorescence 120 arb. units), while after estrogen cytostatic treatment, the maximum accumulation of doxorubicin was observed after 10 min. Doxorubicin concentration in cells incubated with estrogen cytostatic remained practically unchanged over the 30-min observation period, while in control sample not containing estrogen cytostatic, fluorescence decreased by 13.3% over 15 min (mean fluorescence 104 arb. units). This effect of estrogen cytostatic is probably related to its influence on the ATP pump involved in rapid efflux of doxorubicin from the cell. Published data and results of our previous experiments show that antiestrogen cytostatics suppress function of membrane-bound enzymes [1]. These data provide support for our conclusion that antiestrogen cytostatics affect membrane-bound P-glycoprotein.

Our results indicate that antiestrogen cytostatics produce a chemosensitizing effect and increase the sensitivity of resistant MCF-7/R cells to doxorubicin. It can be hypothesized that the chemosensitizing effect of these compounds is realized via modulation of P-glycoprotein.

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