Interaction of bis-β-Chlorethylamine Estrogen Derivatives with Human Ovarian Adenocarcinoma Cells

A. V. Semeikin, V. M. Rzheznikov, O. N. Minailova, Ya. V. Dobrynin, and E. E. Mayatskaya

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Antiproliferative effect of bis-β-chlorethylamine estrogen derivatives on human ovarian adenocarcinoma CaOV cells depends on the dose of the drug. In concentrations of 10^{-5} and 5×10^{-6} M they inhibit, while in a dose of 5×10^{-7} M stimulate cell proliferation. It is assumed that CaOV cells express estradiol receptors. The interaction of these cytostatics with estrogen-binding sites on CaOV cells is demonstrated.

Key Words: *bis-β-chlorethylamines; estrogens; cell culture; estradiol receptors*

Alkylating cytostatics with intrinsic hormonal activity are considered to be promising antitumor drugs. Their molecule consists of the alkylating agent chlorophenacyl and synthetic estrogen analog with estrogen or antiestrogen activities [1,2]. It was anticipated that conjugation of the alkylating group to hormone can modify its distribution in the organism and specifically directed the cytostatic molecules to the target organs, thus potentiating pharmacological effect and reducing toxicity of the antitumor agent [3-6]. The aim of the present study was to compare cytostatic activity and receptor binding of bis-β-chlorethylamine (BC) estrogen derivatives.

MATERIALS AND METHODS

Experiments were carried out on monolayer cultures of human ovarian adenocarcinoma CaOV (Oncology Research Center, Russian Academy of Medical Sciences). The cells were cultured in standard medium 199 supplemented with 10% heat-inactivated fetal calf serum, 100 μg/ml L-glutamine (N. F. Gamaleya Institute of Epidemiology and Microbiology) and 40 μg/ml gentamicin (Ferrein).

Department of Molecular Pharmacology and Radiobiology, Russian State Medical University; Endocrinology Research Center, Russian Academy of Medical Sciences, Moscow

For evaluation of antiproliferative activity, test drugs in a final concentrations of 10^{-5}, 5×10^{-6}, 10^{-6}, and 5×10^{-7} M were added to 96-well plates containing cell monolayer (5×10^5 cells/ml medium). Cells incubated without test drugs served as the control. Proliferative activity was assessed by ^{3}H-thymidine incorporation into DNA. Radioactivity was measured in an Intertechnique SL-30 scintillation β-counter.

Inhibition of ^{3}H-estradiol binding to estrogen-binding sites in CaOV cells (10^6 cells/ml) in the presence of test drugs served as the measure of their affinity to estrogen receptors. ^{3}H-Estradiol (specific activity 37 kBq/mol) was added in a concentration of 5×10^{-9} M (in ethanol). Cell suspension containing a 200-fold excess of test substances was incubated for 30 min at 37°C. Samples without test drugs served as the control.

The inhibition of ^{3}H-estradiol binding to estrogen-binding sites in CaOV cells was calculated as the ratio of radioactivity in the presence to that in the absence of a cytostatic and expressed in percents.

The data were processed using Wilcoxon—Mann—Whitney *U* test.

RESULTS

We compared antiproliferative effect of 6 chlorophenacyl derivatives of estrogen analogs (patent estradiol

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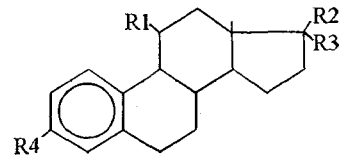

	R1	R2	R3	R4
Estradiol mustard	H	CytO*	H	CytO*
Ia	O_2NO	=O	—	CytO*
Ib	H	CH_3CH_2COO	H	CytO*
Ic	COOH	CH_3CH_2COO	H	CytO*
Id	H	CH_3COO	C≡H	CytO*
Ie	COOH	=O	—	CytO*
If	COOH	=O	—	OH
Ig	O_2NO	=O	—	OH

*CytO — $COXN(CH_2CH_2Cl)_2$; in estradiol mustard X is absent, in Ia-g $X=CH_2C_6H_4$.

Fig. 1. General formula and radicals of synthetic estrogens and their bis-β-chlorethylamine derivatives.

mustard and new compounds Ia, Ib, Ic, Id, and Ie) and 2 steroids without cytostatic group: estradiol (If) and ethynylestradiol (Ig) (Fig. 1).

Estradiol mustard exhibited maximum antiproliferative effect: it 2-fold inhibited cell proliferation in concentrations of 10^{-5}, 5×10^{-6}, 10^{-6} M and 1.2-fold in a concentration of 5×10^{-7} M. This effect can be attributed to the presence of 2 BC groups in its molecule (Table 1).

Cytostatics with single BC group produced similar but less pronounced effect: in a concentration of 10^{-5} M they inhibited cell proliferation 1.2-2-fold, in a concentration of 5×10^{-6} M — 1.1-1.9-fold, in concentrations of 10^{-6} and 5×10^{-7} M — 1.1-1.2-fold.

These findings suggest that human ovarian adenocarcinoma is very sensitive to cytostatics, in particular BC-derivatives.

Effect of Ia, Ib, and Id depended on the dose: they suppressed cell proliferation in concentrations 10^{-5} and 5×10^{-6} M, but in a concentration of 5×10^{-7} M stimulated it 1.15-1.3-fold.

Compounds If and Ig identical by their steroid structure to Ie and Ia, respectively, but containing no cytostatic groups had only little effect on cell proliferation (Table 1).

Estrogen If in a concentration of 10^{-5} M did not stimulate cell proliferation, while its analog Ie suppressed it by 58%. Estrogen Ig significantly increased proliferative activity of cultured cells by 15%, while cytostatic estrogen Ia inhibited it by 27%. Compounds Ia and Ie are derivatives of estradiol and ethynylestradiol, respectively. Estradiol and ethynylestradiol in a concentration of 10^{-5} M stimulated cell proliferation by 21 and 23%, respectively.

These findings suggest that human ovarian adenocarcinoma is sensitive to estrogens. When comparing the effects of estrogens and their BC-derivatives on CaOV cell proliferation, it can be assumed that their estrogen activity inversely correlates with their antitumor effect.

The cytostatic effect of test drug depends on their binding in target cells: a 200-fold excess of estradiol inhibits binding of ^{3}H-17β-estradiol (5×10^{-9} M) in CaOV cells by 15±0.7%, which indicates the presence of estrogen-specific binding sites in these cells. Estra-

TABLE 1. Effect of Estrogens and Their BC-Derivatives on ^{3}H-Thymidine Incorporation into Human Ovarian Adenocarcinoma Cells (% of Control Taken as 100%, *M*±*m*, *n*=6)

Concentration	Estradiol	Ethynyl-estradiol	Estradiol mustard	Ia	Ib	Ic	Id	Ie	If	Ig
10^{-5}	121±2*	123±2*	41±3*	73±7*	73±7*	79±7*	84±5*	42±2*	102±8*	115±1*
5×10^{-6}	120±4*	121±2*	44±2*	76±5*	85±4*	89±7*	88±4*	56±2*	90±5*	100±9
10^{-6}	94±7	109±5*	56±5*	102±20	96±15	95±4*	93±12	98±17	97±19	92±8*
5×10^{-7}	88±9	105±3*	86±7*	127±5*	120±3*	99±13	112±5*	102±17	83±9*	65±15

Note. *$\alpha\leq5$ compared with the control.

diol mustard exhibited the highest affinity for estrogen receptor. The binding of ^{3}H-17β-estradiol in the presence of estradiol mustard was decreased by 13.4±0.5%. Compound Ia inhibited binding of tritiated estradiol by 7.2±0.2%, Ib — by 10.5±0.3%, Ic — by 12.3±0.7%, Id — 11.5±0.7%, and Ie — 12.7±0.3%.

Correlation analysis revealed a strong inverse correlation between the antiproliferative effect of estrogen BC-derivatives and the proliferation-stimulating effect of original estrogens (r=-0.938), i.e., the higher estrogen activity of the agent, the weaker its antiproliferative effect. On the other hand, the antitumor effect only weakly correlated with drug affinity for estrogen-binding sites in CaOV cells (r=0.31). The relationship between the antiproliferative effect of the test agents and the combination of proliferation-stimulating effect of their steroid components and their affinity for estrogen receptors was characterized by the highest correlation coefficient (r=0.95, α<0.1).

Correlation analysis showed that compounds with low estrogen activity and high affinity for estrogen receptors produce most potent antitumor effect.

Thus, antiproliferative activity of the test estrogen cytostatics depends on their dose. In a concentration of 10^{-5} M these agents inhibit cell proliferation. Estradiol mustard exhibits maximum antiproliferative effect, which can be attributed to the presence of 2 BC groups in its molecule. Human ovarian adenocarcinoma CaOV cells express specific estrogen binding sites (probably estrogen receptors). The test estrogen cytostatics specifically interact with estradiol receptors and inhibit binding of tritiated estradiol. These is a multiple correlation (r=0.95) between antiproliferative activity of the cytostatic, proliferation-stimulating activity of its steroid component in CaOV cells, and its affinity for estrogen receptors. This correlation suggests that the most potent antitumor effect is produced by agents with high affinity for estrogen binding sites in combination with low estrogen activity.

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