Effects of Synthetic bis-β-Chloroethylamine Estrogen Derivatives on Proliferation of Skin Sarcoma Cells

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 129, No. 6, pp. 695-697, June, 2000
Original article submitted October 8, 1999

The effects of four new synthetic bis-β-chloroethylamine-containing estrogens and known cytostatic agents chlorophenacyl and estradiol mustard were compared on monolayer cultures of transformed L-929 fibroblasts (from murine skin sarcoma). The drugs within the concentration range of 10^{-5}-5×10^{-7}M inhibited proliferation of cultured cells by 67%. Chlorophenacyl displayed the least antiproliferative activity (15% inhibition at 10^{-5} M). Steroid nucleus introduced into the molecule enhanced antiproliferative activity of test drug in comparison with chlorophenacyl, probably due to accumulation of the hormone-cytostatic molecules in cells. Estradiol had no effect on proliferative activity of L-929 cells, and no specific estrogen-binding sites were found in cultured transformed fibroblasts. The antiproliferative effect of hormone-cytostatics on this culture is not mediated via specific interactions with estrogen receptors.

Key Words: *cytostatic estrogens; cell culture; transformed fibroblasts*

Alkylating agents, derivatives of natural steroids, are widely used in clinical practice [3,4]. For instance, a bis-β-chloroethylamine (BCE) estradiol derivative is the active component of Estracyt used in the therapy of prostate cancer [5-8]. Alkylating derivatives of synthetic steroids displaying a broad spectrum of hormonal and antihormonal effects are less known.

In this work, we studied C ring-transformed estrogens containing a BCE group. Some of these substances exhibit estrogen, while others possess antiestrogen activity [9]. Subcutaneous injections of these substances in a dose of 1 mg/kg for 10 days to C57Bl mice with C37 skin sarcoma inhibited tumor growth by 98-99%. The mechanism of cytostatic effects of these compounds is unknown. There is evidence that introduction of a steroid to the alkylating group of a cytostatic drug changes its distribution in the body, increases specificity of the cytostatic effect, and reduces its toxicity [5-8]. In this work, we compared the antiproliferative effects of synthetic estrogens Ia, Ib, Ic, and Id (Fig. 1 and Table 1) containing a BCE group, estradiol mustard, and chlorophenacyl on transformed murine L-929 fibroblasts. Compounds Ia and Ib belong to the group of ethinyl estradiol, whereas Ic and Id are estrone derivatives. To reveal specific estrogen binding sites on cultured cells that can be responsible for receptor-mediated effects of these compounds, we measured estradiol binding to fibroblasts. In parallel experiments, the proliferative response of cultured cells to estradiol was studied.

MATERIALS AND METHODS

Experiments were performed on monolayer cultures of transformed mouse L-929 fibroblasts. The cells were cultured in medium 199 supplemented with 10% ther-heat-inactivated embryonic calf serum, 100 μg/ml L-glutamine (N.F. Gamaleya NIIEiM), and 40 μg/ml gentamicin (Ferein).

Proliferative responses of cultured cells to the hormone-cytostatic, chlorophenacyl, and estradiol were

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0007-4888/00/0006-592$25.00

evaluated by adding test compounds in final concentrations of 10^{-5}, 5×10^{-6}, 10^{-6}, and 5×10^{-7} M to cell monolayer in 96-well plates (5×10^5 cells per ml medium). Control tests were run without adding test substances. Cell proliferation was estimated by ^{3}H-thymidine incorporation into DNA (radioligand binding assay). The radioactivity of each sample was measured on an Intechnique SL-30 scintillation β-counter [2].

Specific binding of estrogens to cultured fibroblasts was assayed as described elsewhere [1]. The significance of differences was estimated by Wilcoxon—Mann—Whitney test.

RESULTS

Compounds Ia and Id produced the most pronounced antiproliferative effect. In a concentration of 10^{-5} M, these compounds 3- and 2.6-fold inhibited cell proliferation, respectively, and in a concentration of 5×10^{-6} M they produced a 2.5-fold inhibitory effect. Other compounds caused similar but weaker effects: 2-fold inhibition of cell proliferation in a concentration of 10^{-5} M and 1.5-1.6-fold inhibition in a concentration of 5×10^{-6} M. In a concentration of 5×10^{-7} M the test substances were practically ineffective. Chlorophenacyl in a concentration of 10^{-5} M 1.2-fold inhibited cell proliferation. Estradiol produced a slight antiproliferative effect on L-929 fibroblasts in a concentration of 10^{-5} M and had no effect on cell proliferation in other concentrations.

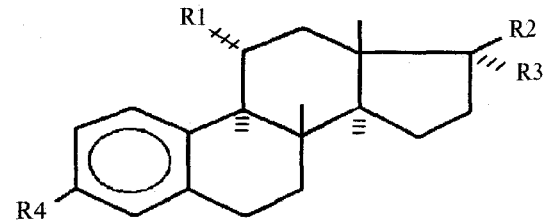

Fig. 1. General formula and positions of radicals in synthetic estrogens and their bis-β-chloroethylamine derivatives. Radicals are presented in Table 1.

Estradiol binding assay was performed to reveal estrogen receptors (ER) in cultured cells. No specific estradiol binding sites were found in L-929 cells: binding of ^{3}H-estradiol in the presence of a 100-fold excess of cold hormone was 97% of the control (in the absence of excess estradiol).

Steroids bearing alkylating groups produced a dose-dependent inhibitory effect on fibroblast proliferation (by 11-67% in a concentration range of 5×10^{-7}-10^{-5} M). These data suggest that L-929 cells are sensitive to BCE derivatives. Chlorophenacyl displayed

TABLE 1. Radical of Estradiol Mustard and BCE Derivatives of Synthetic Estradiols

Radical	Estradiol mustard	BCE derivatives			
		Ia	Ib	Ic	Id
R1	H	OH (a)	CH_3COOH (a)	COOH (b)	COOH (a)
R2	CytO*	CH_3COOH	CH_3COOH	=O	=O
R3	H	C=CH	C=CH	—	—
R4	CytO*	CytO*	CytO*	CytO*	CytO*

Note: *CytO=CO-X-N$(CH_2CH_2Cl)_2$; in BCE derivatives, X stands for $CH_2C_6H_9$, X is absent in estradiol mustard.

TABLE 2. Effects of BCE Estrogen Derivatives on ^{3}H-Thymidine Incorporation into DNA of L-929 Cells (% of Control, $M\pm m$, n=3)

Compound	Molar concentration			
	10^{-5}	5×10^{-6}	10^{-6}	5×10^{-7}
Estradiol mustard	54±3*	67±1*	68±5*	100±7*
Ia	33±3*	40±5*	87.0±0.5*	83±8*
Ib	49±1*	60±8*	77±2*	89±4*
Ic	55±1*	61±1*	98±9*	100±13
Id	39±5*	40±6*	76±2*	100±3*
Estradiol	90±4*	92±12	100±4*	100±3*
Chlorophenacyl	85±1*	84±7*	100±1*	100±17

Note: *p<0.05 compared to the control.

the lowest antiproliferative activity (15% inhibition for drug concentration of 10^{-5} M).

Estradiol did not stimulate proliferation of L-929 cells, and these cells contained no estrogen specific binding sites. Hence, the antiproliferative effects of hormone-cytostatics on this culture were not mediated by their specific interactions with ER.

Thus, introduction of chloroethylamine into estrogen molecules potentiated antiproliferative activity of the alkylating group. Compounds Ia and Id producing the most potent antiproliferative effects are the most promising for further studies. Estrogen BCE derivatives produce strong antiproliferative effects on cells devoid of specific estrogen binding sites; therefore, these compounds can be regarded as potential universal antitumor agents.

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