PHARMACOLOGY AND TOXICOLOGY

Binding of bis-β-Chloroethylamine Derivatives of Synthetic Estrogens to Proteins: Dependence on the Chemical Structure

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We studied binding of 10 new bis-β-chloroethylamine derivatives of synthetic estrogens of different chemical structure to estradiol receptors in cytosolic fraction of breast carcinoma tissue and to blood plasma proteins. 11α-Derivatives of estrone and ethynylestradiol with bis-β-chloroethylamine radical at the 3-position were most potent, while 11β-substances with the cytostatic residue in this position less effectively competed with labeled estradiol for estradiol receptors. Estrone derivatives with cytostatic residue at the 3-position bound primarily to serum albumin. Ethynylestradiol derivatives with cytostatic residue at the 3-position of the steroid nucleus bound to plasma globulins. Cytostatic radical at the 11-position changed spatial conformation of estrogen cytostatics and they lost their ability to interact with estradiol receptors and blood plasma proteins.

Key Words: *estrogen cytostatics; estradiol receptors; blood plasma proteins*

The synthesis of low toxic antitumor drugs with directed action from synthetic steroids is based on the existence of protein receptors that specifically bind molecules with hormonal or antihormonal activity in target tissues.

The interaction of hormonal cytostatics with protein receptors determines their accumulation in tumors arising from target tissues and selectivity of their antitumor effect [4,6]. Previous studies showed that general toxicity and transport of pharmacological compounds depend on the interaction of substances with blood plasma proteins [2]. During synthesis of hormonal cytostatics it is impossible to predict their ability to interact with protein molecules and estimate their efficiency and toxicity. Here we evaluated whether the interaction of bis-β-chloroethylamine derivatives of synthetic estrogens with proteins depends on their chemical structure. The understanding of these relationships would allow us to perform directed synthesis of hormonal cytostatics.

MATERIALS AND METHODS

We studied estrogens transformed by the C-ring with bis-β-chloroethylamine-containing fragments (Fig. 1) possessing different antitumor activity against transplanted estrogen-sensitive tumors (rat breast carcinoma), and exhibiting low general toxicity (*in vivo* tests). 11α- and 11β-Derivatives of estrone and ethynylestradiol were studied. The bis-β-chloroethylamine residue was attached at the 3-position (8 compounds) or 11-position (2 compounds).

We determined the ability of compounds to compete with ^{3}H-17β-estradiol for estradiol receptors in the cytosolic fraction of human breast carcinoma tissue. The samples were obtained from the Department of Prognosis for the Efficiency of Conservative Antitumor Therapy (P. A. Gertsen Moscow Research Institute of Oncology). We studied the interaction of estrogen cytostatics with blood plasma proteins from

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healthy donors (Diagnostic Laboratory, ASTR Hospital). Chlorophenacyl (Embichin) and 17β-estradiol were used as reference drugs.

The ability of compounds to compete with ^{3}H-17β-estradiol for estrogen receptors in the cytosolic fraction of human breast carcinoma tissue was determined by the method of M. Schneider [7] with modifications (competitive activity). {2,4,6,7}^{3}H-17β-estradiol (50 nM, in ethanol, Sigma), test compounds (10^{-5} M in ethanol), and 100 ml cytosol with estradiol receptor concentration of 50 fmol/mg protein were placed in incubation tubes. Binding of the labeled hormone was recorded using a β-radiometer and expressed in cpm. Competitive activity of compounds was determined by displacement of labeled estradiol (%) and calculated by the following formula:

$$CA = T\text{-}NSB/T \times 100\%,$$

where *CA* is competitive activity, *T* is the mean total binding of labeled hormone (without competitor), and *NSB* is the mean nonspecific binding in similar aliquots (with competitor).

Fluorescence of the probe 1-anilinonaphthalene-8-sulfonate (ANS) in human serum albumin (HSA, Sigma) solution and human blood plasma was recorded on a MPF-3 spectrofluorometer (Hitachi) at excitation and emission wavelengths of 377 and 465 nm, respectively. To study the effect of test compounds on binding of ANS to blood plasma proteins, these substances were added to the incubation medium containing 2 ml HSA or human blood plasma in a concentration of 10^{-5} M, 0.1 M phosphate buffer (pH 7.4), and 20 µl 5×10^{-5} M ANS to final concentrations of 10^{-10}, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5} M [1]. Fluorescence intensity was measured in the presence (I) and absence of test compounds (I_0).

RESULTS

Bis-β-chloroethylamine derivatives of synthetic estrogens competed with ^{3}H-17β-estradiol for estradiol receptors in the cytosolic fraction of breast carcinoma tissue (Table 2).

The competitive ability of bis-β-chloroethylamine estrogen derivatives to bind to estradiol receptors depended on the chemical structure and position of radicals in the steroid nucleus. The maximum binding to estrogen receptors was demonstrated by compounds containing α-radicals at the 11-position of the steroid nucleus and cytostatic residues at the 3-position (Po-728, Po-731, Po-714, Po-715, and Po-716). Substances with the 11β-configuration containing cytostatic residues at the 3-position demonstrated lower affinity for estradiol receptors (Po-732, Po-800, and Po-801). Compounds with bis-β-chloroethylamine-containing radicals at the 11-position (Po-725, Po-729) were least potent in competing with estradiol. Chlorophenacyl did not bind to estradiol receptors.

We compared the interaction of bis-β-chloroethylamine estrogen derivatives, estradiol, and chlorophenacyl with blood plasma proteins. Substances differing by chemical structure were divided into 3 groups (Fig.

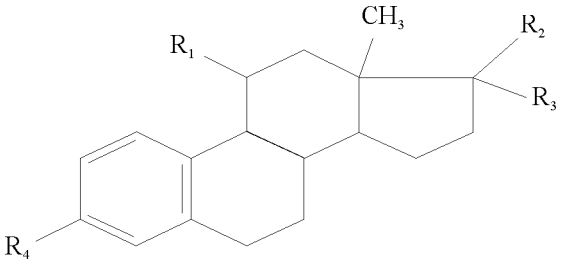

Fig. 1. General formula and position of radicals in bis-β-chloroethylamine derivatives of transformed estrogens. The composition of radicals is shown in Table 1.

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

Derivative	R1	R2	R3	R4
Po-714 (11α)	OH	CH_3COO	C≡CH	CytO
Po-715 (11α)	HCOO	CH_3COO	C≡CH	CytO
Po-716 (11α)	CH_3COO	CH_3COO	C≡CH	CytO
Po-725 (11β)	CytO	CH_3COO	C≡CH	OH
Po-728 (11α)	HCOO	=O	—	CytO
Po-729 (11β)	CytO	=O	—	CH_3COO
Po-731 (11β)	HCOO	=O	—	CytO
Po-732 (11β)	O_2NO	=O	—	CytO
Po-800	H	CH_3CH_2COO	H	CytO
Po-801	H	CH_3COO	C≡CH	CytO

Note. CytO group: $COXN(CH_2CH_2Cl)_2$, where *X* is $CH_2C_6H_4$.

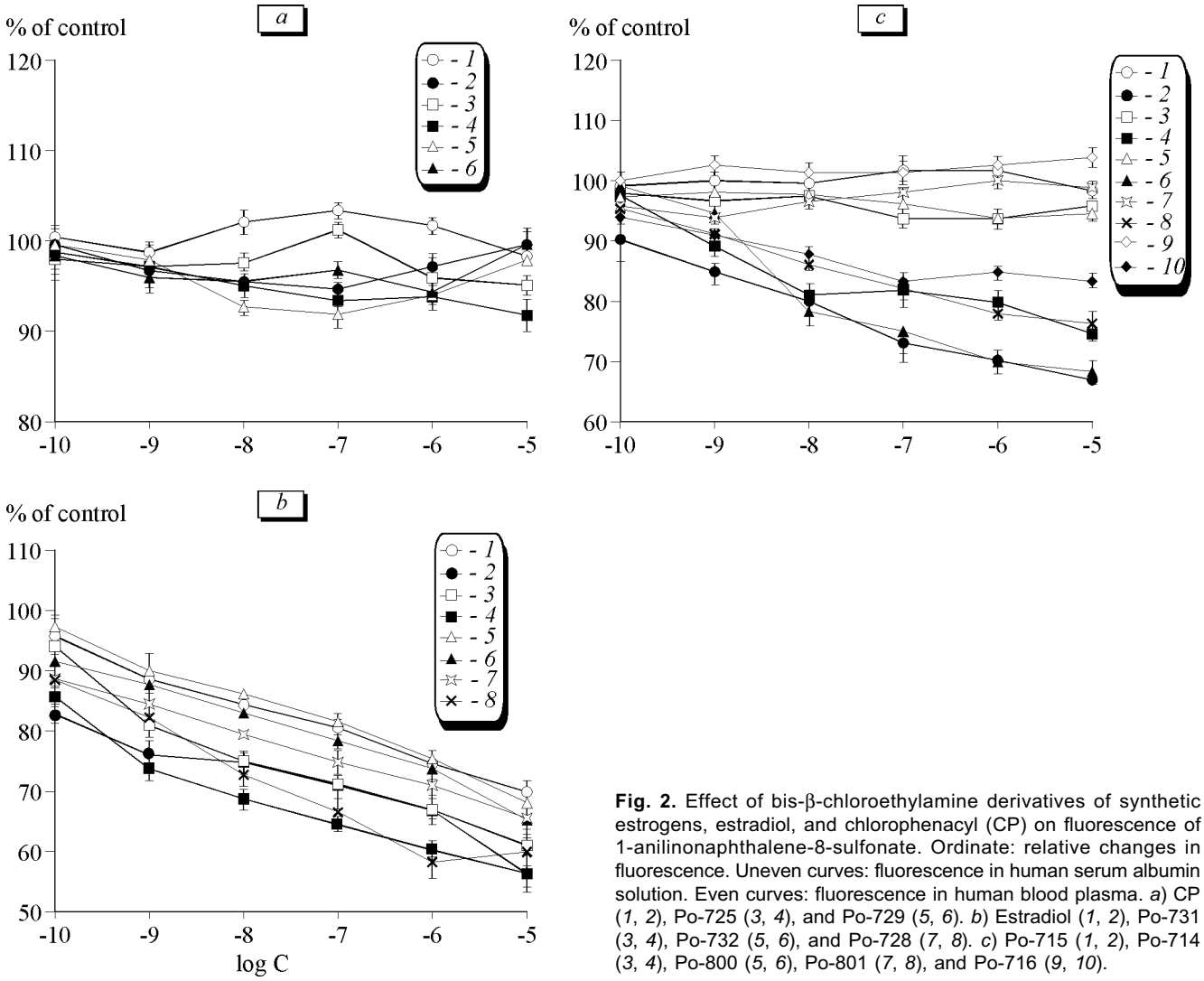

Fig. 2. Effect of bis-β-chloroethylamine derivatives of synthetic estrogens, estradiol, and chlorophenacyl (CP) on fluorescence of 1-anilinonaphthalene-8-sulfonate. Ordinate: relative changes in fluorescence. Uneven curves: fluorescence in human serum albumin solution. Even curves: fluorescence in human blood plasma. *a*) CP (*1*, *2*), Po-725 (*3*, *4*), and Po-729 (*5*, *6*). *b*) Estradiol (*1*, *2*), Po-731 (*3*, *4*), Po-732 (*5*, *6*), and Po-728 (*7*, *8*). *c*) Po-715 (*1*, *2*), Po-714 (*3*, *4*), Po-800 (*5*, *6*), Po-801 (*7*, *8*), and Po-716 (*9*, *10*).

2). Ethynylestradiol and estrone derivatives (Po-725 and Po-729, respectively) containing bis-β-chloroethylamine radicals at the 11-position had no effect on fluorescence intensity in the solution of HSA and whole plasma. Probably, these compounds did not interact with blood transport proteins. Moreover, we did not observe binding of chlorophenacyl to blood plasma proteins (Fig. 2, *a*).

Estrone derivatives with the cytostatic residue at the 3-position (Po-728, Po-731, and Po-732) similarly decreased the intensity of ANS fluorescence in HSA solution and whole plasma (Fig. 2, *b*). The data suggest that these compounds bound primarily to HSA.

Ethynylestradiol derivatives with cytostatic residue at the 3-position of the steroid nucleus (Po-714, Po-715, Po-716, Po-800, and Po-801) did not modulate the intensity of ANS fluorescence in HSA solution, but reduced this parameter in the whole plasma (Fig. 2, *c*). Our findings suggest that these compounds interact with globulins specifically binding sex steroids, but not with HSA. The possibility of synthesizing compounds with affinity for globulins increases their value in directed therapy of hormone-dependent neoplasms. Published data show that globulins interacting with sex steroids have specific binding sites on the plasma membranes of hormone-dependent tissues. Globulins interact with these sites and promote accumulation of bound steroids and hormonal cytostatics in specific tissues [3,5].

Our results show that attachment of steroid components to the bis-β-chloroethylamine radical increases affinity of cytostatics for binding sites in estrogen receptors and blood plasma proteins. Binding of estrogen cytostatics to proteins depends on spatial conformation of their molecules. Introduction of large bis-β-chloroethylamine-containing radicals into the 11-position of steroids yields bulky spatial structures preventing the interaction of estrogen cytostatics with the protein molecule. Configuration of radicals at the 11-position (α- or β- conformation) affects affinity of

compounds for estradiol receptors. Chemical structure of radicals at the 11-position determines the interaction between estrogen cytostatics and blood plasma proteins. The keto group and ethynyl radical are responsible for the interaction of compounds with serum albumin (nonspecific blood transport system) and globulins that specifically bind sex steroids, respectively. These results indicate that the estrogen component determines binding of estrogen cytostatics to protein molecules. It should be emphasized that lateral chains at the 11-position and 17-position play the major role in this process. Understanding of structural criteria reflecting affinity of the compound for estradiol receptors and blood plasma proteins would allow us to perform directed synthesis of substances with low toxicity and high selectivity to estrogen receptor-positive neoplasms. Our findings suggest that Po-714, Po-715, and Po-716 with high affinity for cytosolic estradiol receptors and blood plasma globulins will be tropic in relation to estrogen receptor-positive tumors. Further studies of these compounds are required to synthesize medicinal preparations for the therapy of estrogen-dependent tumors.

TABLE 2. Displacement of ^{3}H-17β-Estradiol (5×10^{-5} M) from the Complex of Estradiol Receptors in the Cytosolic Fraction of Human Breast Carcinoma with bis-β-Chloroethyl-amine Derivatives of Synthetic Estrogens (200-Fold Excess, *M*±*m*, *n*=2)

Compound	^{3}H-E_2 displacement, %
E_2	49.2±5.3
Po-728	56.6±3.4*
Po-731	50.1±3.2*
Po-714	48.7±5.6*
Po-715	46.6±3.1*
Po-716	44.3±3.1*
Po-732	33.0±2.5*
Po-800	31.2±2.4*
Po-801	29.9±2.2*
Po-725	8.0±4.1*
Po-729	14.9±3.4*
CP	0.3±0.2

Note. *$p<0.05$ compared to binding of ^{3}H-17β-estradiol in the absence of test compounds.

REFERENCES

1. Yu. A. Vladimirov and G. E. Dobretsov, *Fluorescent Probes in Studies of Biological Membranes* [in Russian], Moscow (1980).
2. P. V. Sergeev, P. A. Galenko-Yaroshevskii, and N. L. Shimanovskii, *Essays on Biochemical Pharmacology* [in Russian], Moscow (1996), pp. 54-66.
3. R. Frairia, N. Fortunati, and F. Fissore, *J. Endocrinol. Invest*., **15**, No. 8, 617-619 (1992).
4. J. Muntring and G. Jensen, *Prostate*, **21**, No. 4, 287-295 (1992).
5. G. Noe, Y. C. Cheng, M. Dabike, and H. B. Croxatto, *Biol. Reprod*., 47, No. 6, 970-976 (1992).
6. K. Pavelic, I. Zgradic, and K. Pavelic, *J. Cancer. Res. Clin. Oncol*., **117**, 244-248 (1991).
7. M. R. Schneider and E. von Angerer, *J. Med. Chem*., **25**, 1070 (1982).