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Acknowledgements—We thank Ms Joan Greenwood for measuring polyamines and Mrs Wendy Merkey for secretarial assistance. This work was supported by a grant from the National Cancer Institute, POI CA40011.

Eur J Cancer, Vol. 26, No. 5, pp. 608–610, 1990.
Printed in Great Britain

0277–5379/90$3.00 + 0.00
Pergamon Press plc

Lack of binding of gestodene to estrogen receptor in human breast cancer tissue

K. Pollow, M. Juchem, H.J. Grill, W. Elger, S. Beier, K. Schmidt-Gollwitzer and B. Manz

Competition studies with progesterone and estradiol receptors of human myometrial tissue as well as of mammary cancer tissue showed that gestodene bound with high affinity to the progesterone receptor, as did other synthetic and natural progestogens. However, gestodene did not bind to the estradiol receptor. The relative binding affinities of all tested synthetic and natural ligands showed no organ-specific differences and no differences between neoplastically transformed and normal tissues.
Eur J Cancer, Vol. 26, No. 5, pp. 608—610, 1990.

INTRODUCTION

GESTODENE, a 19-nortestosterone derivative, is an effective progestogen [1]. Iqbal *et al.* [2, 3] reported significant binding of gestodene to the estradiol receptor of human malignant tissue but no binding to this receptor in normal breast tissue or endometrium. Such findings are surprising because they demonstrate that a progestogen such as gestodene, which is structurally related to levonorgestrel and its optical isomer *d*-norgestrel, displaces estradiol from its receptor. Therefore, we have investigated the binding of gestodene to the estradiol receptor of normal and neoplastic breast tissue as well as that of normal myometrium.

MATERIALS AND METHODS

(17α-methyl-[^{3}H])-R5020 (specific activity 3.22 TBq/mmol), (2,4,6,7-[^{3}H](N))-estradiol (specific activity 3.44 TBq/mmol) and unlabelled R5020 were purchased from New England Nuclear (Dreieich, F.R.G.). Unlabelled Org2058 was obtained from Amersham Buchler (Braunschweig, F.R.G.). Tritiated as well as unlabelled gestodene were supplied by Schering. Other unlabelled steroids used were obtained from Serva (Heidelberg, F.R.G.). The buffer was PENG (10 mmol/l KH_2PO_4, 10 mmol/l K_2PO_4, 1.5 mmol/l EDTA, 3 mmol/l NaN_3, 5 mmol/l monothioglycerol, 10% glycerol), pH 7.5. Dextran-coated charcoal was 0.5% 'Norit A' and 0.05% dextran 'T400' in PENG buffer.

Breast cancer tissue was obtained at operation. Samples were confirmed histologically and then estradiol and progesterone receptor concentrations were measured [4]. Only receptor-positive tumors were used and these were pooled and stored in liquid nitrogen until assayed. Myometrial tissue was obtained after hysterectomy because of myoma uteri and prolapsed uterus. Some of the sample was sent for histological examination and the rest was used for receptor assay.

To prepare cytosol, the frozen tissue samples were minced and after the addition of cold PENG buffer, were homogenized (Ultra Turrax). The homogenate was centrifuged at 105,000 *g* at 4°C for 30 min. The clear supernatant was used. Protein concentrations were measured by the Lowry method. Malignant breast or normal uterine cytosols (100 µl) were incubated with [^{3}H]steroids (50 µl, final concentration 8 nmol/l) with increasing concentrations (50 µl, final concentration 10^{-10} to 10^{-5} mol/l) of various competitor steroids for 4 h at 4°C. Unbound steroids were adsorbed by incubating with 0.5 ml DCC suspension for 10 min at 4°C. After centrifugation (10 min, 1500 *g*, 4°C), 0.5 ml of the supernatant was withdrawn and counted for radioactivity. The relative binding affinities (RBA) were calculated with the method of Korenmann [5].

Table 1. Relative binding affinities (%) of gestodene compared with other steroid hormones at progestin and estrogen receptors of human breast cancer tissue

Steroid	Progesterone receptor: [^{3}H]R5020	[^{3}H]gestodene	Estradiol receptor: [^{3}H]estradiol
Gestodene	90	100	< 0.1
R5020	100	50	< 0.1
Org2058	250	65	< 0.1
Medroxyprogesterone acetate	130	30	< 0.1
Levonorgestrel	110	90	< 0.1
Progesterone	45	9	< 0.1
DHT	< 0.1	< 0.1	< 0.1
Diethylstilbestrol	< 0.1	< 0.1	85
Estradiol	< 0.1	< 0.1	100

*Reference steroid. DHT = dihydrotestosterone.

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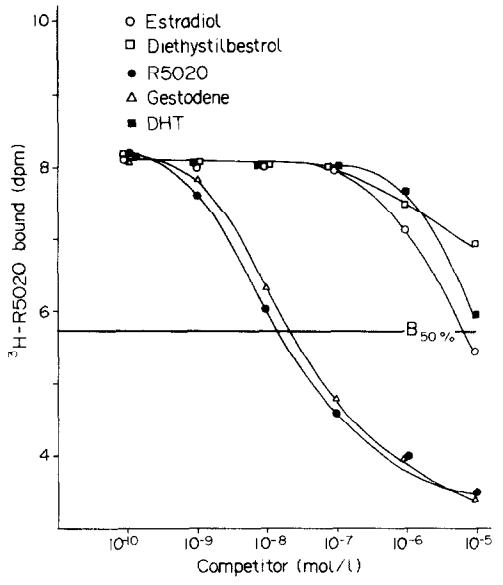

Fig. 1. Binding of [^{3}H]R5020 to cytoplasmic progesterone receptor of human mammary carcinoma.

RESULTS

Table 1 and Figs 1–3 summarize the results of the competitive behavior of gestodene compared with various synthetic and natural steroids at the cytosolic progesterone and estradiol receptors of breast cancer tissue. Compared with R5020, a potent ligand for progesterone receptors that does not bind to serum proteins [6], gestodene at the progesterone receptor from mammary tissue had an RBA value of 90%. With [^{3}H]gestodene as the reference steroid all other progestogens could also displace label from its binding site on the progesterone receptor to various extents. Furthermore, DHT, estradiol and diethylstilbestrol bound at the progesterone receptor in a way typical for all progestogens including 19-nortestosterone derivatives, i.e. lack of binding compared with ^{3}H-labelled R5020 or gestodene.

Binding studies with progesterone and estradiol receptors of myometrial tissue showed that gestodene bound with high

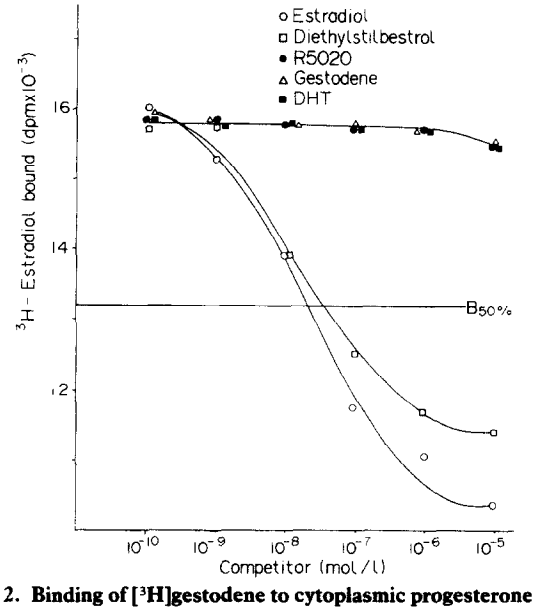

Fig. 2. Binding of [^{3}H]gestodene to cytoplasmic progesterone receptor of human mammary carcinoma.

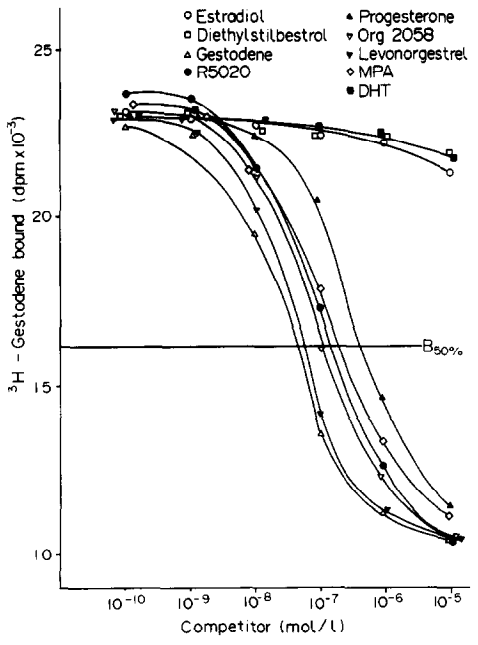

Fig. 3. Binding of [^{3}H]estradiol to cytoplasmic estradiol receptor of human mammary carcinoma.

affinity to the progesterone receptor, as did other synthetic and natural progestogens, but (as for some of the other progestogens tested) gestodene did not bind to the estradiol receptor from uterine cytosol (Table 2).

Comparison of the RBA values of all tested ligands showed no organ-specific differences and no differences between neoplastically transformed and normal tissues at the respective progesterone and estradiol receptors.

DISCUSSION

Iqbal *et al.* [2, 3] suggested that binding of gestodene to the estrogen receptor from human mammary cancer tissue but not to that from normal breast tissue or normal endometrium indicated structural differences between the receptors in normal and neoplastic breast tissue. Furthermore, their evidence indicated that gestodene may be of clinical value as an antiestrogen in the management of breast cancer not by decreasing the estradiol receptor content in the malignant breast cell but by direct competition at the receptor with estradiol.

Table 2. Relative binding affinities (%) of gestodene compared with other steroid hormones at progestin and estrogen receptors of human myometrial tissue

Steroid	Progesterone receptor: [^{3}H]R5020	Progesterone receptor: [^{3}H]gestodene	Estradiol receptor: [^{3}H]estradiol
Gestodene	85	100	< 0.1
R5020	100	25	< 0.1
Org2058	350	33	< 0.1
Medroxyprogesterone acetate	115	12	< 0.1
Levonorgestrel	120	75	< 0.1
Progesterone	40	2	< 0.1
DHT	< 0.1	< 0.1	< 0.1
Diethylstilbestrol	< 0.1	< 0.1	110
Estradiol	< 0.1	< 0.1	100

Our results showed that gestodene behaves at the receptor level in the manner typical for progestogens in general and 19-nortestosterone derivatives in particular. Gestodene bound with high affinity to the progesterone receptor, whereas specific displacement of [^{3}H]estradiol by unlabelled gestodene was not detected at the estradiol receptor. This applied in both human myometrial and breast cancer tissue, and thus contradicts the earlier studies [2, 3], in which gestodene was said to be three times more effective in displacing [^{3}H]estradiol from cytosolic and nuclear estradiol receptors in malignant breast tissue than the natural ligand.

We used the charcoal-dextran method to separate receptor-bound and free steroid, whereas Iqbal *et al.* [2, 3, 8] used a two-tier column microassay for affinity immobilization of the receptor and the steroid bound to it. No experience with this method has been reported in other laboratories. It is possible that a tumor-associated protein from specific breast cancers (whether present in the serum or in the tumor cell) was absorbed by the separating material with the method used in Iqbal's laboratory. This protein might be able to bind estradiol but at the same time allow gestodene to displace estradiol competitively because of high affinity. To what extent this protein is to be classified as a receptor requires further clarification. Gestodene does bind with high affinity in a similar way to estradiol to sex hormone binding globulin. This is typical for 19-nortestosterone derivatives [9], including gestodene.

Thus, the earlier statement [2, 3] that besides its gestagenic potency, gestodene may also be of clinical value as an anti-estrogen in the management of malignant breast disease, similar to tamoxifen [10], as a competitor for estradiol bound at its receptor is too far-reaching. It is not disputed that progestogens are generally anti-estrogens, since endogenous progestogens are naturally involved in the down-regulation of estradiol receptors [1].

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