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Estrogen and Antiestrogen Binding Sites in Desmoid Tumors*

C.L. LIM,† M.J. WALKER,‡ R.R. MEHTA and T.K. DAS GUPTA

Division of Surgical Oncology, Department of Surgery, University of Illinois College of Medicine at Chicago, Cook County Hospital, the West Side Veterans Administration Hospital, and the Hektoen Institute for Medical Research, Cook County Hospital, Chicago, IL 60612, *U.S.A.*

Abstract—*Clinical and experimental evidence suggests a role for estrogen in the natural history of desmoid tumors (DT). Antiestrogen (tamoxifen) has been used empirically in some patients with significant tumor regression. To further investigate the mechanism of hormonal influence on desmoid tumors we initially characterized the cytosol estrogen receptor (ER) and antiestrogen binding sites (AEBS) in microsomal fractions of* 15 *cases of DT. Biopsy specimens were obtained from nine female and six male patients. ER assay was determined in cytosol* (105,000 ***g***) *and the AEBS was detected in the microsomal fraction* (7000 ***g*** *for* 20 min) *by a DCC assay technique. ER was present in 33% of DT assayed* (5/15), *with equal incidence in males and females. Receptor content in female patients was higher than in male patients* (26.52 ± 16 vs 10.82 ± 8.32 fmol/mg protein). *Dissociation constant* (K_d) *range* (0.44–3.97 nM) *was well within the values seen in other estrogen target tissues. The AEBS were detected in* 79% *of the cases. The mean binding value was 236.7 ± 170.2 fmol/mg protein.* K_d *values were between* 0.39 *and* 5.97 nM. *ER settled predominantly in the 4S region and AEBS settled in the* 5–5.5*S region in a* 5–20% *sucrose gradient. AEBS was detected in seven patients with negative ER. No correlation between ER and AEBS contents was observed. Competition studies revealed minimal binding with either DEX, DHT, R*5020, *and R*1881, *but partial binding with tamoxifen in cytosol and estradiol in microsomal fractions. ER and AEBS assays may be of prognostic significance in the natural history of these tumors.*

INTRODUCTION

DESMOID tumors (aggressive fibromatosis) are rare tumors of fibrous origin [1–3]. Clinical correlates suggest that steroidal hormones may have a role in the natural history of these tumors. It is predominantly seen in female patients of child-bearing age [4–7], and regression of these tumors has been associated with menopause [5]. More recently, Kinzbrunner *et al.* [8] noted that high-dose tamoxifen led to complete relief of pain and a decrease in the size of a desmoid tumor. Similarly, Waddell *et al.* [9] attained significant tumor regression in patients with desmoid tumors following treatment with tamoxifen and nonsteroidal antiinflammatory drugs.

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Reprint requests and correspondence should be addressed to: M.J. Walker, M.D., Division of Surgical Oncology Room 372, CME Building, University of Illinois College of Medicine at Chicago, 840 South Wood Street, Chicago, IL 60612, U.S.A.

As an initial step in investigating the mechanism of hormonal influence on desmoid tumors, we studied the incidence and binding characteristics of cytosol estrogen receptor and subcellular fraction AEBS of 15 cases of desmoid tumors.

MATERIALS AND METHODS

Specimens were obtained from 15 patients (nine females, six male) who were operated on for either primary or recurrent desmoid tumors during the period April 1978 to April 1985. Tumor tissues were immediately divided into 1-cm portions, frozen in liquid nitrogen, and stored at −80°C until receptor analysis. Before freezing, a portion of each specimen was processed for examination by both light and electron microscopy.

Specimens for assay were weighed while still frozen and pulverized in a tissue pulverizer. All subsequent steps were performed at 0–4°C. The pulverized tissue was homogenized with two 10-sec bursts in a polytron homogenizer separated by a 30-sec cooling period in the following buffer system: estrogen buffer (buffer A) — 10 mM Tris-HCL, 5 mM EDTA, 10 mM sodium molybdate, 12

mM monothioglycerol (v/v), 10 mM dithiotreitol, 10% glycerol (v/v), pH 7.4. For AEBS assay (buffer B) — 0.2% BSA was added to Buffer A. The homogenate was centrifuged at 7000 ***g*** for 20 min and the pellet discarded. The supernatant was recentrifuged at 105,000 ***g*** for 60 min to obtain cytosol for estrogen receptor analysis and the microsomal pellet resuspended in Buffer B for AEBS assay.

Cytosol and subcellular fractions were diluted to 1–2 mg protein/ml for receptor analysis by a modification of dextran-coated charcoal (DCC) [10,11] or 5–6 mg/ml for sucrose density gradient (5–20%) analysis. Cytosol protein was determined according to the method of Bradford [12]. For estrogen receptor (ER) assessment, cytosols (200 μl) were incubated for 16 hr at 4°C over a concentration range of 0.05–6 nM [^{3}H] estradiol in parallel with 100-fold molar excess of DES. For antiestrogen binding sites (AEBS), 200 μl of the microsomal fraction were incubated for 16 hr at 4°C over a concentration range of 0.05–6 nM [^{3}H] tamoxifen in parallel with 100-fold excess radioinert tamoxifen. The bound was separated from the free ligand by the DCC technique. Data were analyzed according to the method of Scatchard [13].

For sucrose density gradient analysis tumor cytosols (250 μl) were also layered on 4.6 ml of 5–20% sucrose density gradients, made up in an appropriate buffer following a 4-hr parallel incubation with labelled (5 nM [^{3}H]-estradiol or [^{3}H]-tamoxifen) and 100-fold excess of radioinert ligands. Free steroid was removed during a 15-min incubation with a DCC pellet at 4°C (1500 rpm for 15 min), and cytosols were centrifuged for 16 hr at 285,000 ***g*** in a S.W. 50.1 rotor (Beckman Instruments). The gradient tubes were punctured, and 10-drop fractions were collected and counted in a 5-ml aqueous counting scintillation cocktail at an efficiency of 33%. ^{14}C-BSA was used as internal standard with a sedimentation constant of 4.6S for the determination of the sedimentation coefficient according to the method of Martin and Ames [14].

Competition studies

Cytosol fractions were diluted to 1–2 mg protein/ml; 5 nM [^{3}H] estradiol was incubated with 200 μl cytosol in the presence or absence of 100 × cold competitor for 16 hr at 4°C. The DCC technique was used for separation of free from bound ligand. Data were expressed as the percentage of the specific binding of [^{3}H] estradiol in the presence of 100 × ethinyl estradiol. For AEBS competition, 200 μl was incubated with 5 nM [^{3}H] tamoxifen in the presence or absence of different molar concentrations of cold competitors for 16 hr at 4°C. DCC was used for separation. The data were expressed as the percentage of the specific [^{3}H] tamoxifen bound. The competitors used in both studies were dihydrotestosterone (DHT), dexamethasone (DEX), promegestone (R5020), methyltrienolone (R1881), estradiol (E_2), and tamoxifen (T).

RESULTS

Table 1 summarizes the incidence and distribution of estrogen and AEBS in 15 patients with desmoid tumor. There were six males and nine females, with a total ER incidence of 33%. ER incidence was similar in both female (3/9) and male (2/6) patients. The mean ER levels were higher in the female patients than in the male (26.52 vs 10.82 fmol/mg cytosol protein). Tumors from 14 patients were available for evaluation of AEBS. The overall incidence of AEBS was 78.5%, 7/9 females (77.7%) and 4/5 males (80%) had detectable binding. There was no difference in AEBS between males (255.7 fmol/mg cytosol protein) and females (225.9 fmol/mg protein). Table 2 sumarizes the relation of the presence of cytosolic estrogen receptor and presence or absence of AEBS.

There were five female patients and two male patients with no ER in the cytosol fraction, but AEBS was present in the subcellular fraction. Among the four patients who had both ER and AEBS, the mean AEBS level was 11 times the mean ER level (166 vs 14.13 fmol/mg protein). There were two patients with neither estrogen nor AEBS (one male, one female) and one female with estrogen cytosol receptor and no subcellular binding for tamoxifen. There was no correlation between the cytosol ER level and microsomal AEBS level. The mean dissociation constant (K_d) for AEBS was 2.9 × the K_d for ER (4.18 vs 1.44 nM). There was no correlation between the location of the tumor and the presence of binding for estrogen or tamoxifen.

Scatchard analysis of the binding data (Fig. 1) showed a single class of specific high-affinity saturable receptors for estrogen and tamoxifen. To further characterize the estrogen and AEBS, sucrose density gradient ultracentrifugation analyses were performed on selected tumor samples.

Figure 2 shows the sucrose density gradient for cytosol binding of [^{3}H] 17-β-estradiol. It settled predominantly in the 4S region under low salt conditions.

Figure 3 shows a representative result of the gradient analysis of [^{3}H] tamoxifen binding to microsomal fraction of desmoid tumor. The [^{3}H] tamoxifen alone was characterized by a 5–5.5S peak under low salt conditions which was suppressible by 100-fold molar excess of unlabelled tamoxifen. This 5–5.5S peak was not suppressed by preincubation of the microsomal fraction with 100-

Table 1. *Clinical characteristics and binding parameters of estrogen and antiestrogen binding sites in* 15 *desmoid tumors*

Pt	Sex	Age	Estrogen receptor fmol/mg prot.	K_d (nM)	A.E.B.S. fmol/mg prot.	K_d (nM)	Location
1	F	21	44.67	3.97	Neg.	–	Left posterior thigh
2	F	33	20.9	0.54	381.74	5.67	Right deltoid muscle
3	F	32	Neg.	–	130.5	2.16	Right upper arm
4	F	36	Neg.	–	201.2	5.97	Left side of neck
5	F	40	14	0.94	109	3.86	Dorsum of right foot
6	F	21	Neg.	–	457.7	7.5	Abdominal wall
7	F	65	Neg.	–	8.7	1.18	Left post chest wall
8	F	42	Neg.	–	Neg.	–	Right suprascapular
9	F	24	Neg.	–	292.2	7.5	Right shoulder
10	M	19	4.93	0.44	140.48	3.9	Abdominal wall
11	M	31	Neg.	–	Neg.	–	Left upper back
12	M	52	16.7	1.3	35.2	0.39	Right upper back
13	M	59	Neg.	–	514	4.53	Left retroperitoneum
14	M	63	Neg.	–	333	3.33	Abdominal wall
15	M	35	Neg.	–	N.A.		Retroperitoneal

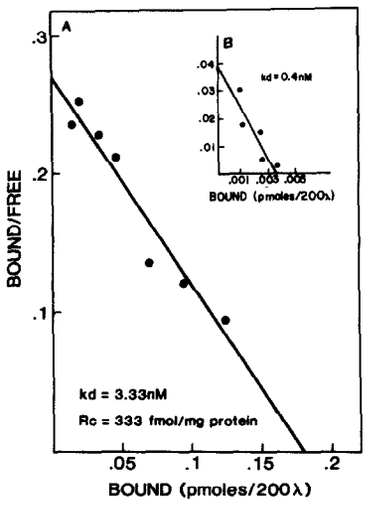

Fig. 1. *Scatchard Analysis of* [^{3}H] *tamoxifen binding in microsomal fraction of desmoid tumor from Patient* 14. *Inset — Scatchard Analysis of* [^{3}H] 17-β-*estradiol binding in cytosol fraction of desmoid tumor from Patient* 10.

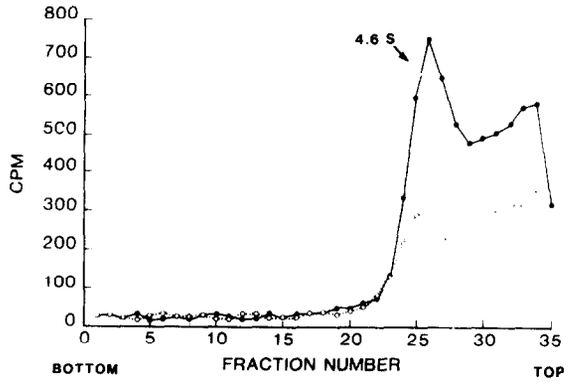

Fig. 2. *Five to twenty per cent sucrose density gradient of* [^{3}H] 17-β-*estradiol binding in the cytosol fraction of desmoid tumors.* ^{14}C *labelled albumin was used as internal standard.*

Table 2. *Incidence and correlation of estrogen and antiestrogen binding sites in* 14 *desmoid tumors*

	n	Sex Male	Female
ER^+ $AEBS^+$	4	2	2
ER^+ $AEBS^-$	1	0	1
ER^- $AEBS^+$	7	2	5
ER^- $AEBS^-$	2	1	1

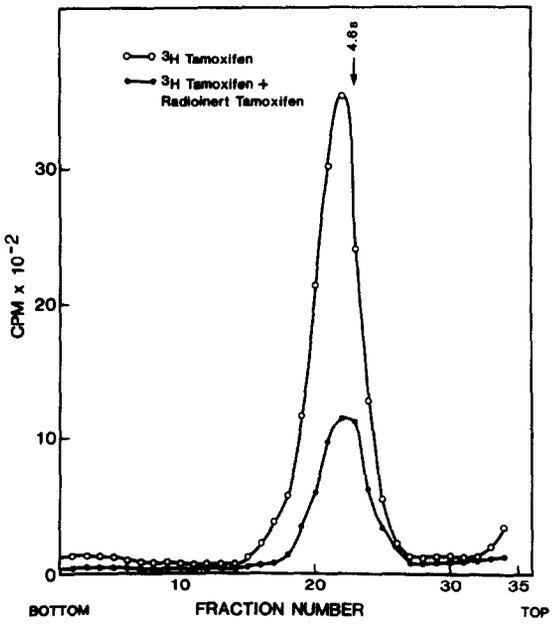

Fig. 3. *Five to twenty per cent sucrose density gradient of* [^{3}H] *tamoxifen binding to microsomal fraction of desmoid tumor.* C^{14} *labelled albumin was used as internal standard.*

fold molar excess of radioinert estradiol for 30 min before adding [^{3}H]-tamoxifen. AEBS is reported to be a membrane bound microsomal protein [15,16].

Whether the 5–5.5S peak observed in the present study represents the true sedimentation constant of this protein or is just an aggregation of membrane fragments with bound [^{3}H] tamoxifen is not clear at the moment.

Specificity of bindings of [^{3}H] estradiol to tumor cytosol and [^{3}H] tamoxifen to microsomal fraction were achieved by the competition of various steroids for binding of [^{3}H] estradiol and [^{3}H] tamoxifen (Fig. 4). Tamoxifen competed moderately well with [^{3}H] estradiol binding in the tumor cytosol (74%). While estradiol was partially competitive (35%) with [^{3}H] tamoxifen in the microsomal fraction. DEX, DHT, R5020, and R1881 were weak competitors for [^{3}H] Tamoxifen and [^{3}H] estradiol binding.

DISCUSSION

There is clinical and experimental evidence suggesting a possible hormonal influence on desmoid tumor development and progression. Lipschutz and co-workers [17] described the formation of fibrous tumors histologically similar to desmoids in the abdominal organs, anterior abdominal wall, and thorax of guinea pigs after prolonged estrogen administration and the prevention of these tumors by the administration of testosterone, progesterone, and desoxycorticosterone [18–20]. High-dose estrogen treatment in a man with prostate cancer induced abdominal desmoid four years later which subsequently regressed after hormone interruption and orchiectomy [21]. Pregnancy seems to stimulate the growth of desmoid tumor [4, 5, 22] and regression of this tumor has been associated with menopause [5] and onset of menstruation [23].

The finding of a cytosolic receptor for estrogen in this group of tumors represents the first step in determining a putative role for estrogen in the biology of these tumors. In our original report of steroid receptors in soft tissue sarcomas, we found no estrogen binding in two desmoid tumors [24]. Havry *et al.* [25] demonstrated ER in three of four patients with desmoid tumors, although the receptor levels were low (range 1.9–4.6 fmol/mg protein). Our overall incidence of cytosolic estrogen binding is lower than 33% in a larger series of patients and our receptor levels are generally higher. The affinity constant is well within the values seen in other estrogen target tissue and is specific for estrogen. Although the presence of a cytosolic estrogen receptor does not imply a biologic role for estrogen it is necessary for the mechanism of estrogen as it is understood today.

Tamoxifen, a nonsteroidal antiestrogen, is thought to mediate its effects through the specific ER of hormonally responsive tissue [26–29]. More

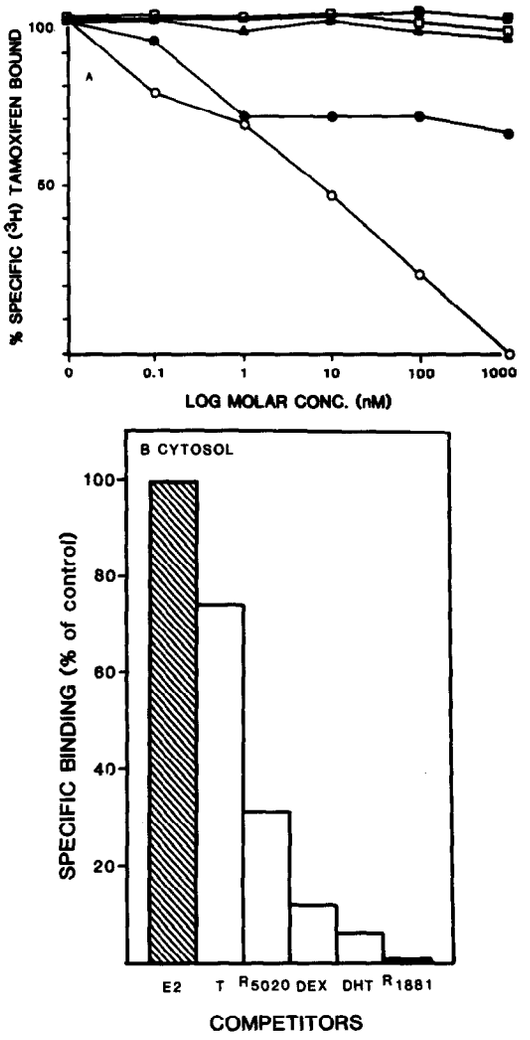

Fig. 4. (a) *Competition for* [^{3}H] *tamoxifen binding sites by various steroids in desmoid tumors.* (○—○ *Tamoxifen,* ●—● *DES,* ▲—▲ *R5020,* ■—■ *R1881,* □—□ *DEX*).
(b) *Competition for ER in cytosol fractions of desmoid tumors.*

recently, Sutherland *et al.* [15] described an antiestrogen binding site distinct from ER and suggested this binding site might be important in modulating or mediating the action of the antiestrogen. Sudo *et al.* [16] found these sites to fractionate in the microsomal or subcellular fraction of rat uterus and only very small amounts are detected in the high-speed cytosol. We have adopted Sudo's procedure and detected a 79% incidence of binding for tamoxifen. Four tumors which were ER-negative were found to have high levels of AEBS in the subcellular fraction. This might explain responses to tamoxifen [8, 9] when an overall incidence and concentration of estrogen receptor is low for an estrogen-responsive tissue.

We have demonstrated cytosol estrogen receptor and microsomal antiestrogen binding sites in a group of desmoid tumors. This is a preliminary step toward understanding the role of estrogens and antiestrogens in the biology of these tumors.

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