Interaction of Tamoxifen with Estradiol Cytosol Receptors of Uterine Myoma and Histologically Unaltered Myometrium

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Uterine myoma is an estradiol-dependent hyperplastic process developing on a background of chronic hyperestrogenia in women of reproductive age. The treatment of uterine myoma of interstitial and subperitoneal localization includes hormonal preparations with androgenic activity, estrogen-gestagens, and synthetic progestins, in order to obtain an antiestrogenic effect [4]. However, nonsteroid synthetic antiestrogenic preparations, such as tamoxifen, are not used in the treatment of uterine myoma.

This paper was devoted to a study of the relative ability of tamoxifen to bind with the estradiol cytosol receptors [3,5,6] in human myoma and adjacent unaltered myometrial tissue, in order to assess the possibility of using this nonsteroid estrogen in the treatment of myoma as well as to elucidate the mechanism of action of tamoxifen.

MATERIALS AND METHODS

Leiomyoma nodules and specimens of surrounding normal myometrium were obtained in the course of surgical intervention. The tissue specimens were processed for 2 hours postoperation or stored in liquid nitrogen (-70°C) for no longer than 2 days. For the preparation of homogenates, tumor tissue was cut into small pieces with a scalpel and placed in a mortar, which was cooled with liquid nitrogen. The tissue was frozen in liquid nitrogen and ground to a powder, which was transferred to the centrifuge tube and extracted with 6-8 volumes of TED buffer at pH 7.4, containing 10 mM Tris-HCl (Merck, Germany), 1.5 mM EDTA (Sigma, USA), 0.5 mM dithiothreitol (Koch-Light Laboratories, England), and 0.3% sodium azide (Merck, Germany), with the addition of 10% glycerol (superfine grade, USSR). The homogenate was clarified by centrifugation for 15 min at 1500 g (0-4°C) in a Janetski K-24 centrifuge. The cytosol fraction was obtained by centrifugation of the supernatant at 105,000 g, 0-4°C, for one hour in a UP-65M ultracentrifuge. The relative binding ability of tamoxifen was assessed by a widely accepted method after Schneider and von Angerer [8] with the following modifications: 5 nM ^{3}H-estradiol, 10^{-8}-10^{-4} M tamoxifen, and 100 µl cytosol with a receptor concentration of not less than 20 fM per mg protein were placed into incubation tubes. As a control of nonspecific binding, 17β-estradiol (Merck, Germany) in a concentration of 2 µM was used as a competent ligand. After 18-20-hour incubation at 0-4°C the unbound hormone was sedimented by the addition of a suspension of activated charcoal Norit A (Serva, Germany) coated with Dextran T-70 (Serva, Germany) and subsequent centrifugation at 1500 g for 15 min (0-4°C). 100 µl of

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TABLE 1. Relative Binding Ability of Tamoxifen to Estradiol Receptors in Human Myometrium ($M \pm m$)

Tissue specimen	Number of observations	Cytosol ER (fM per mg protein)	Relative binding ability (%)
HUM from myoma-affected uterus	12	18.5±4.5	0.07±0.009
Myoma node	12	34.8±8.5*	0.36±0.09*

Note. Asterisk denotes values significantly different from those in HUM ($p<0.05$).

supernatant from each tube was transferred to scintillation flasks containing 5 ml of dioxane-based scintillation fluid, and measured in an SL-30 liquid scintillation counter (Intertechnique, France). The relative binding ability of tamoxifen was calculated according to the formula $[E_2]/[I]$, where $[E_2]$ is the concentration of estradiol in the assay system and [I] is the concentration of tamoxifen required for 50% displacement of receptor-bound estradiol. Protein content was measured after Lowry [7]. The results were statistically evaluated using the Student t test [1].

RESULTS

The concentration of cytosol estradiol receptors in the myomatous tissue and surrounding myometrium, as well as the levels of relative binding ability of tamoxifen to the estradiol receptors (ER) are shown in Table 1.

It can be seen that the cytosol ER content in the myomatous nodes exceeds that of the histologically unaltered myometrium (HUM), which is in agreement with the data in the literature [2,4].

The figures in Table 1 illustrate the competitiveness of tamoxifen to estradiol regarding ER binding in the myomatous nodes. The revealed ability of tamoxifen to effectively displace estradiol at its receptors in myomatous tissue may be considered as justification for the use of tamoxifen in the treatment of myoma.

REFERENCES

1. A. Wentzel, *Probability Theory* [Russian translation, 1969], p. 325.
2. E. M. Vikhlyaeva and L. N. Vasilevskaya, *Uterine Myoma* [in Russian], Moscow (1981), p. 30.
3. E. N. Mineeva, *Farmakol. Toksikol.*, № 6, 26-27 (1990).
4. *Steroid Hormone Receptors in Human Tumors* [in Russian], Ed. L. S. Bassalyk, Moscow (1987), pp. 222-224.
5. P. V. Sergeev and E. N. Mineeva, *Farmakol. Toksikol.*, № 1, 4-8 (1990).
6. D. M. Ignar-Trowbridge, K. G. Nelson, Kim A. Ross, *et al.*, *J. Steroid Biochem.*, 39, № 1, 131-132 (1991).
7. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265-275 (1951).
8. M. R. Schneider and E. von Angerer, *J. Med. Chem.*, **25**, 1070-1077 (1982).