Acta Oncologica Vol. 31, No. 2, pp. 143–146, 1992

AGONISTIC AND ANTAGONISTIC EFFECTS OF ANTIESTROGENS IN DIFFERENT TARGET ORGANS

LAURI KANGAS

Antiestrogens block by definition specifically the actions of estrogens. In the classical uterotropic assay in immature rodents, where estrogens cause fluid retention and cell proliferation, triphenylethylenes have also species-specific estrogen-like (agonistic) effects. 4-hydroxylated triphenylethylenes have in general less estrogenic properties than unhydroxylated ones, and ICI 164,384 has no estrogenic activity in this model. Uterus responds to estrogens by stimulation of cell proliferation. Some other tissues, like breast, liver, and bone respond by regulation of specific protein synthesis. Some of the proteins act as growth factors, and some have unknown functions. The regulation of gene expression is a complex phenomenon: estrogens may turn the responsive gene on or off. Similarly antiestrogens may participate in the gene regulation by mimicking or antagonising estrogen-like actions. This paper summarizes the estrogenic and antiestrogenic effects of classical and new antiestrogens in different tissues.

Key words: Antiestrogens, tamoxifen, toremifene, estrogen target tissues, cell proliferation, hormonal regulation of gene expression.

Acta Oncol., Vol. 31, No. 2, pp. 143–146, 1992.

Estrogens bind to specific estrogen receptors (ER), and as receptor complex to estrogen responsive elements (ERE) in chromatin, where they act as one of the transcription-like factors regulating gene expression (1, 2). Estrogen receptors are found in estrogen target tissues. Other tissues are thought to be non-responsive to estrogens. High ER concentrations are typically found in mammary gland, endometrium, and hypothalamic area, but other tissues like liver, immunoresponsive cells and certain cancer tissues also contain measurable concentrations of ER (3).

Antiestrogens, by definition, block the action of estrogens. However, most known antiestrogens have also agonistic properties which typically are both species and tissue specific (4). One can therefore expect that the regulation of estrogen response is complex. The complexity is due to the interactions of several proteins at the regulatory areas of estrogen responsive genes. Some of these proteins have inhibiting or masking and some have activating effect on transcription (1, 5). In addition, estrogens and antiestrogens indirectly affect cell proliferation and cell metabolism via growth factor stimulation or inhibition or via unknown mechanisms.

Paper presented at the 4th Scandinavian Breast Cancer Symposium, June 3–5, 1991, at Haikko Manor, Porvoo, Finland.
Submitted 11 December 1991.
Accepted 2 January 1992.
Correspondence to: Dr Lauri Kangas, Orion Corporation FARMOS, R&D Pharmaceuticals, P.O. Box 425, SF-20101 Turku, Finland.

Binding of estrogens and antiestrogens to ER and ERE

Typically antiestrogens are bound to ER competitively with estradiol although the binding affinity may vary. The antiestrogen-ER complex is activated by all antiestrogens. Tamoxifen, toremifene, most of their metabolites and derivatives, as well as raloxifene-ER complexes are bound to ERE specifically; apparently to the same site as estradiol-ER complex (6). However, a new completely pure antiestrogen, ICI 164,384, after attaching to ER, prohibits binding of the complex to ERE and causes rapid loss of ER (7, 8).

Table 1

Relative estrogenic potency in the rat uterus of some well-known antiestrogens

Hormone	Relative estrogenicity
Estradiol	+++
Tamoxifen	++
Toremifene	+(+)
4-hydroxytamoxifen	+
Raloxifene	+
Droloxifene	+
ICI 164,384	−

Effects of antiestrogens in the uterus

Mouse and rat are the commonly used target species for studying the estrogen and antiestrogen-like actions of different compounds. Estradiol induces in three days an easily demonstrable uterotropic effect (fluid retention and cell proliferation) in about 20-day-old rodents (9). Pure antiestrogen (e.g. ICI 164,384) inhibits this effect without causing any uterotropic effect. Most known antiestrogens block the uterotropic action but have variable intrinsic estrogenic action as shown in Table 1. Triphenylethylene antiestrogens have more estrogenicity in mice and dogs than in rats. However, the estrogenicity of tamoxifen and toremifene in long-term administration to mice is markedly diminished (10, 11). In humans tamoxifen and toremifene are able to oppose the estrogen-induced vaginal cornification index (12). As in rats, both compounds have weak intrinsic estrogenic effect in the vaginal mucosa of postmenopausal women.

Effects of antiestrogens in the liver

Liver is not considered to be a target organ for estrogens. This is probably true as regards cell proliferation. However, estradiol is known to influence the synthesis of several proteins in the liver; probably the best known of these in human is SHBG, which binds estradiol with high affinity and keeps the concentration of circulating free estradiol low (13). Estrogens also participate in regulation of synthesis of cortisol binding globulin, antithrombin III, ceruloplasmin and plasma renin substrate (14). Diethylstilbesterol (DES), tamoxifen and toremifene increase slightly the serum concentrations of ASAT and ALAT. DES induces clearly ceruloplasmin and plasma renin substrate synthesis, but tamoxifen and toremifene have no effect on these proteins. In the liver tamoxifen and toremifene on the other hand increase the nuclear ER concentrations—an effect which is not seen with DES (14).

In humans tamoxifen and toremifene increase significantly SHBG-concentrations in postmenopausal women, but on the other hand antithrombin III levels decrease (15, 16). There are no available data of pure antiestrogens on the liver protein synthesis.

Long-term toxicity studies in rats have revealed an unexpected carcinogenic potential of tamoxifen. Although tamoxifen and toremifene seem to have similar estrogenicity in the liver (14), this carcinogenic potential was not seen with toremifene (Hirsimäki et al., unpublished study). Tamoxifen but not toremifene, induces production of cytochrome P448-type enzymes in the liver. Oxidative stress, as well as inhibition of cholesterol biosynthesis with accumulation of cholesterol intermediates have been suggested as a mechanism for the carcinogenicity of tamoxifen (17). The latter explanation is highly interesting as the first clinically tested triparanol (MER-29) caused similar effects on cholesterol metabolism (18). Several enzymes of cholesterol biosynthesis are inhibited by antiestrogens and it is tempting to speculate that this effect is not mediated by estrogen receptors (19).

In the liver the effects of estrogens are specific, but diverse. Triphenylethylene antiestrogens have partly antagonistic, partly estrogen-like actions, and partly actions which are not related to estrogens.

Effect of antiestrogens on bone

The physiological effect of estrogens in the bone is most clearly visible during the menopause: formation of the bone does no more increase, but resorption of the bone continues at almost the same rate as premenopausally (20). This changes the balance of bone metabolism towards osteolysis. Similar bone loss is not seen in men which further stresses the importance of estrogens in bone metabolism. By antagonizing estrogen action antiestrogens should increase the bone loss. However, women who receive tamoxifen therapy for breast cancer seem to lose bone density at slower rate or not at all when compared to a control population (21, 22). This refers to an estrogen-like effect of partial antagonists in the bone. Similarly, in long-term toxicity studies in mice tamoxifen and toremifene induce trabecular bone formation (23) and tamoxifen preserves bone in rats after ovariectomy (24).

The bone metabolism is complex: osteocytes take care of the bone formation and osteoclasts of bone resorption. Either of these cells do not have measurable ER, although technical problems may jeopardize receptor determinations in these cells (22). The indirect antiestrogenic effects therefore dominate. Tamoxifen and toremifene increase the secretion of transforming growth factor beta (TGFβ) (25), which stimulates extracellular matrix synthesis and upregulates e.g. collagen type I and PDGF synthesis in the stromal cells of the bone. Similarly insulin-like growth factor (IGF I and II) concentrations are increased by estrogens. These factors stimulate cell proliferation, increase collagen synthesis and decrease collagen degradation (26). The net effect of estrogen stimulation in the bone therefore favors bone formation. On the other hand estrogens may also induce PDGF, which among other effects

stimulates osteoclast activity and bone resorption. It is, however, interesting that PDGF may stimulate TGFβ secretion in bone tissue culture (27). The role of osteoclasts is therefore highly interesting: being capable of producing TGFβ in acidic environment, they may stimulate extracellular matrix synthesis in the stromal cells. The bone saving effect of estrogens in postmenopause is probably stronger (osteoblast activation and increase of bond density) than that of tamoxifen and toremifene (inhibition of resorption probably due to osteoclast inhibition). On the contrary, pure antiestrogens very probably induce bone resorption by blocking all estrogen-like effects.

Immunologic effect of antiestrogens

Autoimmune diseases are more common in females than in males. In autoimmune NZB/NZW female mice testosterone treatment and ovariectomy markedly reduce the signs of the autoimmune syndrome and increase the life span (28). Tamoxifen and toremifene have similar effect (29) when given chronically. The net effect of tamoxifen and toremifene therefore seems to be antiestrogenic in autoimmune mice. However, they do not inhibit NK-cells or their activation by interferon (29), although estradiol clearly suppresses both basal NK-cell activity and inhibits interferon activation. Toremifene and tamoxifen have no major effects on the immunological status of breast cancer patients (30, 31). Estrogens and antiestrogens regulate certain immunological functions. However, more data are needed to make any conclusions about their net effects in breast cancer patients.

The properties of antiestrogens in different tissues are summarized in Table 2.

REFERENCES

1. Gronemeyer H, Green S, Kumar V, Jeltsch J-M, Chambon P. Structure and function of the estrogen receptor and other members of the nuclear receptor family. In: Sheridan PJ, Blom K, Trachtenberg MC, eds. Steroid receptors and disease. New York: Marcel Dekker, 1988: 153–87.
2. Webster NJG, Green S, Jin JR, Chambon P. The hormone binding domains of the estrogen and glucocorticoid receptors contain an inducible transcription activation function. Cell 1988; 54: 199–207.
3. Stumpf WE, Sar M. Autoradiographic localization of estrogen, androgen, progestin, and glucocorticoidsteroid in 'target tissues' and 'nontarget tissues'. In: Pasqualini JR, ed. Receptors and mechanism of action of steroid hormones, Part 1. New York: Marcel Dekker, 1975: 41–84.
4. Jordan VC. Biochemical pharmacology of antiestrogen action. Pharmacol Rev 1984; 36: 245–76.
5. Singh RK, Ruh MF, Butler WB, Ruh TS. Acceptor sites on chromatin for receptor bound by estrogen versus antiestrogen in antiestrogen-sensitive and resistant MCF-7 cells. Endocrinology 1986; 118: 1087–95.
6. Kangas L. Development and biochemical pharmacology of toremifene, antiestrogenic anticancer drug [dissertation]. Turku, Finland: Univ of Turku, 1990.
7. Wakeling AE. Novel pure antiestrogen. Mode of action and therapeutic prospects. Ann NY Acad Sci 1990; 595: 348–56.
8. Gibson MK, Nemmers LA, Beckman WC Jr, Davis VL, Kurtis SW, Korach KS. The mechanism of ICI 164,384 antiestrogenicity involves rapid loss of estrogen receptor in uterine tissue. Endocrinology 1991; 129: 2000–10.
9. Terenius L. Structure-activity relationships of anti-oestrogens with regard to interaction with 17β-oestradiol in the mouse uterus and vagina. Acta Endocrinol 1971; 66: 431–47.
10. Jordan VC, Robinson SP. Species-specific pharmacology of antiestrogens: role of metabolism. Fed Proc 1987; 46: 1870–4.
11. Robinson SP, Parker CJ, Jordan VC. Preclinical studies with toremifene as an antitumor agent. Breast Cancer Res Treat 1990; 16 (Suppl): 9–17.

Table 2

Summary of estrogenic, partial antagonistic and pure antagonistic actions of estradiol and different antiestrogens in different tissues

	Estradiol	Antiestrogen	
		Partial	Pure
Binding to ER	+++	+(+)(+)	+++
Binding of ER – complex to ERE	+++	+++	–
Activation of target genes	+++ or –	+++, ++, + or –	–
Uterotropic effect	+++	+, ++	–
Bone effect	saving, indirect ?	inhibit resorption	induce resorption
Liver	specific protein synthesis induced/inhibited	estrogen-like	?
Immunology	worsens autoimmune symptoms	release symptoms	?

12. Mäenpää J, Söderström K-O, Grönroos M, Taina E, Hajba A, Kangas L. Effect of toremifene on estrogen primed vaginal mucosa in postmenopausal women. J Steroid Biochem 1990; 36: 221–3.
13. Siiteri PK, Murai JT, Hammond GL, Nisker JA, Raymoure WJ, Kuhn RW. The serum transport of steroid hormones. Recent Prog Horm Res 1982; 38: 457–510.
14. Iatropoulos MJ, Williams GM, Kendall ME, Rose DP, Meschter CL, Djordjevic MV. Comparison of 12-month toxicity of tamoxifen and toremifene in rats (abstract). In: Int conference on long-term antihormonal therapy for breast cancer, Walt Disney World Swan, Lake Buena Vista, Florida, USA, 1991; June 30–July 2: p. 14.
15. Kivinen S, Mäenpää J. Effect of toremifene on clinical chemistry, hematology and hormone levels at different doses in heatlhy postmenopausal volunteers: phase I study. J Steroid Biochem 1990; 36: 217–20.
16. Bertelli G, Pronzato P, Amoroso D, et al. Adjuvant tamoxifen in primary breast cancer: influence on plasma lipids and antithrombin III levels. Breast Cancer Res Treat 1988; 12: 307–10.
17. Ahotupa M, Hirsimäki P. The effect of tamoxifen citrate and toremifene citrate on lipid peroxidation, antioxidant enzymes and xenobiotic metabolizing enzymes in rat liver [Abstr. No. 10]. V International Congress of Toxicology. Brighton, England 16–21 July 1989: p. 4.
18. Blohm TR, Kariya T, Laughlin MW. Effects of MER-29, a cholesterol synthesis inhibitor, on mammalian tissue lipids. Arch Biochem Biophys 1959; 85: 250–9.
19. Cypriani B, Tabacik C, Descomps B. Effects of estradiol and antiestrogens on cholesterol biosynthesis in hormone-dependent and -independent breast cancer cell lines. Biochim Biophys Acta 1988; 972: 167–78.
20. Tudtud LA, Christenson RA, Baylink DJ. Adrenal and gonadal steroid in bone disease. In: Sheridan PJ, Blom K, Trachtenberg MC, eds. Steroid receptors and disease. New York: Marcel Dekker, 1988: 369–403.
21. Love RR, Cameron L, Connell BL, Leventhal H. Symptoms associated with tamoxifen treatment in postmenopausal women. Arch Intern Med 1991; 151: 1842–7.
22. Aufdemorte TB, Triplett RG, Holt GR. Sex steroids, osteoporosis, ans osteoarthritis. In: Sheridan PJ, Blom K, Trachtenberg MC, eds. Steroid receptors and disease. New York: Marcel Dekker, 1988: 317–28.
23. Highman B, Roth SI, Greenman DL. Osseous changes and osteosarcomas in mice continuously fed diets containing diethylstilbesterol or 17β-estradiol. JNCI 1981; 67: 653–62.
24. Moon LY, Wakley GK, Turner RT. Dose-dependent effects of tamoxifen on long bones in growing rats: influence of ovarian status. Endocrinol 1991; 129: 1568–74.
25. Coletta AA, Wakefield LM, Howell FV, et al. Anti-oestrogens induce the secretion of active transforming growth factor beta from human fetal fibroblasts. Br J Cancer 1990; 62: 405–9.
26. Sandberg M. Matrix in cartilage and bone development: current views on the function and regulation of major organic components. Ann Med 1991; 23: 207–17.
27. Oreffo RO, Mundy GR, Seydin SM, Bonewald LF. Activation of the bone-derived latent TGF beta complex by isolated osteoclasts. Biochem Biophys Res Commun 1989; 158: 817–23.
28. Ansar Ahmed S, Penhale WJ, Talal N. Sex hormones, immune resonses and autoimmune diseases. Mechanism of hormone action. Am J Pathol 1985; 121: 531–51.
29. Wärri A, Kangas L. Effect of toremifene on the activity of NK-cells in NZB/NZW mice. J Steroid Biochem 1990; 36: 207–9.
30. Valavaara R, Tuominen J, Toivanen A. The immunological status of breast cancer patients during treatment with a new antiestrogen, toremifene. Cancer Immunol Immunother 1990; 31: 381–6.
31. Paavonen T, Aronen H, Pyrhönen S, Hajba A, Andersson L. The effects of anti-estrogen therapy on lymphocyte functions in breast cancer patients. APMIS 1991; 99: 163–70.