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Action Mechanism of Antiestrogens on Uterine Growth in Immature Rats

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ABSTRACT

In the present study, we examined the effects of tamoxifen and LY117018 on various parameters for the estrogenic actions in order to understand the mechanism by which tamoxifen and LY117018 act on the uterine cells in 21-23 day old immature rats.

Tamoxifen and LY117018 stimulated uterine weight and uterine contents of DNA, protein, and peroxidase activity in the absence of estradiol while inhibited above parameters in the presence of estradiol. Both cytosolic and nuclear progesterone receptors were increased by the treatment of tamoxifen and LY117018 as well as estradiol, but estradiol-induced increase in the progesterone receptors were reduced by the treatment of antiestrogens. These effects were enhanced by the multiple injections of antiestrogens. It seemed that tamoxifen was more agonistic than LY117018 but less antagonistic than LY117018, judged by their effects on various parameters for the estrogenic action. The affinities of estradiol, tamoxifen, and LY117018 for the estrogen receptor were 0.17 ± 0.01nM (100%), 1.10 ± 0.01nM (6.3%), and 0.23 ± 0.01nM (77%), respectively. Furthermore, LY117018 was the competitive ligand for the estrogen receptor in dose-related manner but tamoxifen was not. Following estradiol treatment, nuclear estrogen receptor was sharply increased by 1 h, reaching the maximum by 16 h, while tamoxifen and LY117018 slightly increased nuclear estrogen receptor by 1 h and then decreased thereafter.

It is therefore concluded that LY117018 is a competitive antagonist for the estrogen receptor with less estrogenic activity, compared to tamoxifen with low affinity to the estrogen receptor, and tamoxifen may act through other binding site than the estrogen receptor.

Key Words: Estradiol, antiestrogens (tamoxifen and LY117018), agonistic, antagonistic, estrogen receptor

INTRODUCTION

Tamoxifen and LY117018 are the non-steroidal antiestrogens which possess a triphenylethylene-based structure (Furr and Jordan, 1984). These antiestrogens are known to inhibit estradiol-stimulated uterine weight in immature rat, to prevent the binding of ^{3}H-estradiol to the uterus in vivo, and competitively to inhibit the binding of ^{3}H-estradiol to the uterine estrogen receptor in vitro (Jordan, 1982). These findings suggest that the non-steroidal antiestrogens are classic pharmacological antagonists with a mechanism of action that depends on the formation of a drug-estrogen receptor complex with low intrinsic efficacy (Ariens and Simonis, 1964).

Triphenylethylene-type antiestrogens appear to differ from each other in their effects at the nuclear level with respect to activation of RNA polymerase I and II (Kurl and Borthwick, 1980) as well as the length of time that antiestrogen-receptor complex is retained in the nucleus (Katzenellenbogen and Ferguson, 1975). Tamoxifen and LY117018 have widely varying inherent estrogenic (agonistic) properties and are also divergent in their antagonistic action toward estradiol (Black and Goode, 1980, 1981). In particular, these compounds have shown to suppress estrogen-stimulated uterine growth in a varie-

ty of animal species and to inhibit the growth of estrogen receptor-containing human breast cancer cells (Katzenellenbogen *et al.*, 1979; Sutherland and Jordan, 1981). The initial step in blocking estrogenic responses by the non-steroidal antiestrogens has been thought to be the binding of antiestrogen to the estrogen receptor (Koreman, 1970). Much of the experimental data are consistent with the hypothesis that antiestrogens exert their effects through the estrogen receptor system of target cells (Katzenellenbogen *et al.*, 1979; Coezy *et al.*, 1982; Eckert and Katzenellenbogen, 1982).

However, Black and Goode (1981) have demonstrated that LY117018 can block the increase in uterine wet weight by estradiol, but it could not prevent estrogenic action of tamoxifen. It was also found that LY117018 interacts differently with the estrogen receptor compared with tamoxifen (Black *et al.*, 1981). Furthermore, several reports (Sutherland *et al.*, 1980; Sudo *et al.*, 1983) have provided evidence for a binding site for tamoxifen, present in the high speed cytosol fraction, that is distinct from the estrogen receptor. This finding strongly suggests that a triphenylethylene-type of antiestrogen could also produce some estrogenic effects by mechanism other than the estrogen receptor.

The present study was undertaken to compare the agonistic and antagonistic effects of tamoxifen and LY117018 on various parameters for estrogenic action and to examine whether both antiestrogens have different binding characteristics toward estrogen receptors, thereby exerting different action mechanism.

MATERIALS AND METHODS

Materials

Immature female rats aged 21-23 days (Sprague Dawley) were used throughout this study. Uterine tissues were stored at −70°C until analyzed after dissecting free of fat and mesentery, blotting, and weighing.

17 β-estradiol (Sigma, USA) and trans-tamoxifen citrate (ICI, USA) were dissolved in sesame oil and saline, respectively and LY117018 (Eli Lilly, USA) was dissolved in absolute ethanol, adjusting final concentration to 0.5-50μg/0.1ml.

Methods

Determination of peroxidase activity

All procedures were performed at 4°C unless indicated otherwise. The uteri were cut into small pieces with scissors and homogenized (0.5g/ml) in buffer T_{10} (10mM Tris-HCl, pH 7.2) using Polytron PT-10 (10 sec burst at speed setting 6; Janke & Kunkel, W. Germany). The homogenate was centrifuged at 40,000×g for 30 min. Uterine peroxidase was solubilized by homogenizing the 40,000×g pellet in buffer $T_{10}C_{500}$ (10mM Tris-HCl, 500mM $CaCl_2$, pH 7.2), followed by centrifugation at 40,000×g for 30 min, to obtain supernatant ($CaCl_2$ extract) containing most of the peroxidase in tissues. Peroxidase activity was determined at 25°C using guaiacol (Sigma) as a cosubstrate. The reaction mixture (3.0ml) contained guaiacol (13mM) and H_2O_2 (0.33mM) in buffer $T_{10}C_{500}$ and 0.02-1.0ml of sample extract. The increase in absorbance at 470nm resulting from the oxidation of guaiacol was measured in a spectrophotometer (Shimazu, Japan). One enzyme unit was defined as the amount of enzyme required to produce an increase of 1 absorbance unit/min, and the results were expressed as unit/mg protein.

Binding assays for estrogen and progesterone receptor

Uterine tissues (0.5g/ml) were homogenized in TEDMG buffer (10mM Tris-HCl, 1mM EDTA, 1mM dithiothreitol, 20mM sodium molybdate and 20% glycerol, pH 7.4) using Polytron PT-10. The homogenate was centrifuged at 1,000×g for 10 min. The supernatant was then centrifuged at 105,000×g for 1 h to obtain the cytosolic fraction. The 1,000×g pellet was homogenized in a glass homogenizer with a teflon pestle and passed through stainless steel grid (50 mesh) to remove the connective tissue. The filtrate was centrifuged at 1,000×g for 10 min and this process was repeated 3 times. The pellet was designated as nuclear receptor extract.

Binding assay was performed by the methods of Park *et al.* (1986a, 1986b). Scatchard analysis was carried out to determine the binding affinity and receptor concentration. 200μl of cytosolic and nuclear fractions (2-3mg protein/ml) were incubated with ^{3}H-estradiol (0.5-4nM) or ^{3}H-R5020 (0.5-4nM) at 4°C for 18 h with or without a 100-fold excess of radioinert DES or progesterone. To determine competitive binding of antiestrogens with estrogen receptor, cytosol (2-3mg protein/ml) was incubated with 4nM ^{3}H-estradiol in the presence of various concen-

trations of radioinert estradiol, trans-tamoxifen or LY117018, at 4°C for 18 h. At the end of incubation, unbound ligands were removed by adding 0.5ml of 0.5% dextran-coated charcoal (0.5% charcoal and 0.05% dextran T-70) suspension before incubation at 4°C for 15 min. The tubes were centrifuged at 1,500×g for 5 min. The radioactivity in the supernatant was counted in the liquid scintillation spectrometer (Beckman LS 5000, Beckman Instruments, USA). The nuclear suspension was incubated with ^{3}H-estradiol or ^{3}H-R5020 at 4°C for 18 h. At the end of incubation, unbound ligands were removed by adding 2 ml buffer and centrifugation at 1,500×g for 5 min 3 times. The pellet was incubated with 2 ml absolute ethanol for 12 h and centrifuged at 1,500×g for 15 min. The radioactivity in the supernatant was counted in a liquid scintillation spectrometer. The specific binding was obtained by substracting the non-specific binding from the total binding, and expressed as pmol/uterus.

Determination of DNA and protein concentration

The DNA concentrations were measured by modification of Burton method (1956) with calf thymus DNA (Sigma) as a standard. The protein concentrations were measured by Lowry method (1951) with bovine serum albumin (Sigma) as a standard.

Statistical analysis

The data were analysed by the one-way ANOVA with t-test and significant difference was declared for $p<0.05$.

RESULTS

Effects of estradiol and antiestrogens on uterine weight and uterine contents of DNA and protein

As shown in Figure 1, single injection of estradiol (50μg) significantly increased uterine weight of immature female rats ($p<0.001$). The treatment of tamoxifen or LY117018 (50μg) also significantly increased uterine weight although the increase was not as great as that was shown with estradiol. However, this increase was greater after the treatment of 100μg for 2 days. In contrast, simultaneous treatment of antiestrogens with estradiol for 2 days significantly decreased estradiol-stimulated uterine weight ($p<0.001$).

The treatment of estradiol or antiestrogen showed a similar effect on protein and DNA contents in

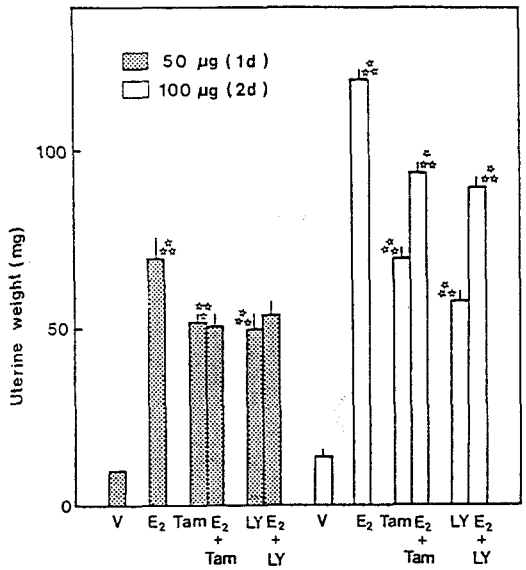

Fig. 1. Effects of estradiol, tamoxifen, and LY117018 on the uterine weight of immature rats. Each bar represents the mean ± SEM of 6 animals. Vehicle (V); estradiol (E_2); trans-tamoxifen citrate (Tam); LY117018 (LY)
*** $p<0.001$; compared with vehicle or estradiol-only.

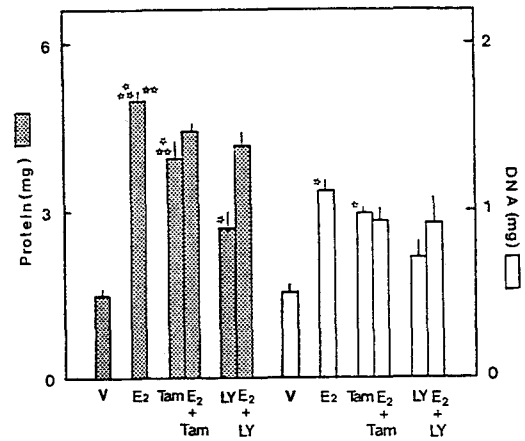

Fig. 2. Effects of estradiol and antiestrogens on the uterine contents of protein and DNA in immature rats. Each bar represents the mean ± SEM of 6 animals.
* $p<0.05$, *** $p<0.001$; compared with vehicle.

uterine tissues (Fig. 2). Protein contents were significantly increased by the treatment of estradiol ($p<0.001$), tamoxifen ($p<0.001$) or LY117018 ($p<0.05$) for 2 days. Estradiol and tamoxifen pro-

duced significant increases in DNA contents ($p<0.05$). However, simultaneous treatment of antiestrogens with estradiol decreased uterine contents of protein and DNA, compared with the estradiol-only treated group. These results indicate that antiestrogens exert partial estrogenic responses in the absence of estradiol, while in the presence of estradiol, antiestrogens antagonize the action of estradiol.

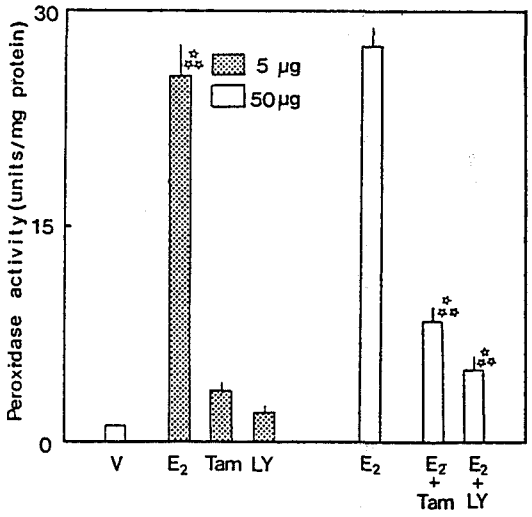

Fig. 3. Effects of estradiol and antiestrogens on uterine peroxidase activity in immature rats. Each bar represents the mean ± SEM of 6 animals.
*** $p<0.001$; compared with vehicle or estradiol-only.

Effects of estradiol and antiestrogens on uterine peroxidase activity

Administration of estradiol (10μg/2 days) showed 23-fold increase in uterine peroxidase activity, compared with the vehicle-treated group, while tamoxifen and LY117018 (10μg/2 days) slightly increased uterine peroxidase activity (Fig. 3). Concomitant treatment of antiestrogens with estradiol (100μg/2 days) significantly decreased estradiol-stimulated peroxidase activity ($p<0.001$, Fig. 3). Tamoxifen or LY117018 decreased estradiol-stimulated peroxidase activity up to 73% or 81% respectively.

Binding characteristics of estradiol and antiestrogens toward uterine estrogen receptor

By employing optimal conditions for estrogen receptor assay (Park *et al.*, 1986a) established in our laboratory, saturation curve and Scatchard plot were constructed by saturating cytosolic and nuclear estrogen receptors with ^{3}H-estradiol (Fig. 4). Cytosolic and nuclear estrogen receptors were saturated with 4nM ^{3}H-estradiol. Binding affinity (Kd

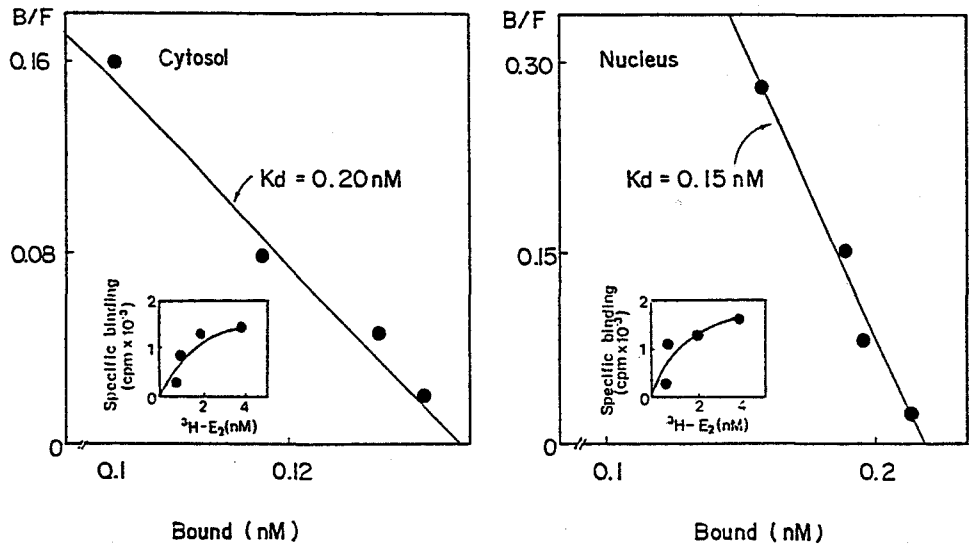

Fig. 4. Saturation curve (insert) and Scatchard analysis for estrogen receptors of immature rat uterus. Receptors were incubated with various concentrations of ^{3}H-estradiol at 4°C for 18 h.

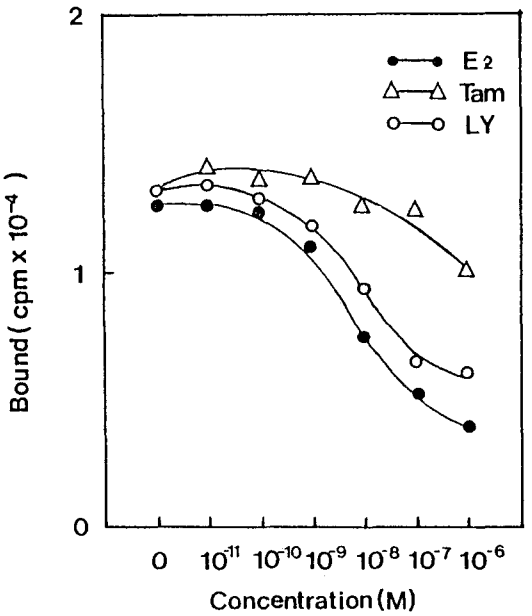

Fig. 5. Competitive binding of antiestrogens with cytosolic estrogen receptors of immature rat uterus. Cytosol was incubated with the radioinert competitor and 4nM of ^{3}H-estradiol for 18 h at 4°C.

Table 1. Binding affinities of estrogen and antiestrogens for uterine estrogen receptor in immature rats

	Kd value (nM)	relative value (%)
Estradiol	0.177 ± 0.01	100
Tamoxifen	1.108 ± 0.015	6.3
LY117018	0.230 ± 0.018	77.0

value) and binding capacity of cytosolic receptor were 0.2nM and 138 fmol/ml respectively while those of nuclear estrogen receptor were 0.15nM and 212 fmol/ml respectively.

Binding affinity of estradiol, tamoxifen, and LY117018 for the uterine estrogen receptor were determined by competitive binding assay. As seen in Fig. 5, the order of competitive effectiveness was estradiol > LY117018 > tamoxifen. This order was paralleled with their affinities for the estrogen receptor (Table 1). Kd values of estradiol, LY117018 and tamoxifen were 0.177 ± 0.01nM, 0.23 ± 0.018nM, and 1.108 ± 0.015nM respectively, indicating that binding affinities of LY117018 and tamoxifen are only 77% and 6.3% of binding affinity of estradiol (100%) for

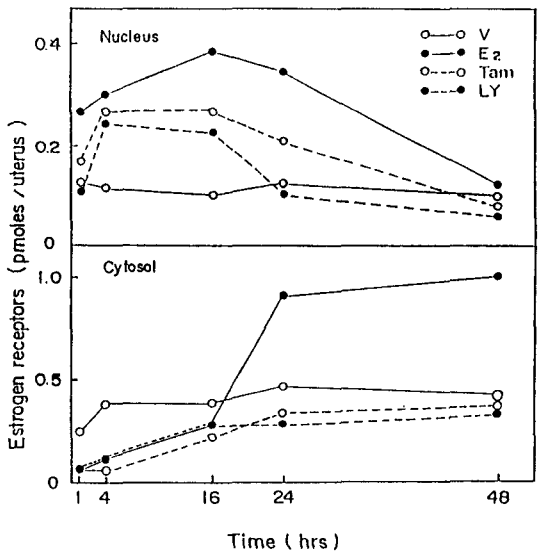

Fig. 6. Time course of effects of estradiol and antiestrogens on estrogen receptors of immature rat uterus.

estrogen receptor. These results suggest that LY117018 is the competitive ligand for estrogen receptor while tamoxifen may act through other binding site than estrogen receptor.

Effects of estradiol and antiestrogens on uterine estrogen receptor

Estrogen receptor concentrations were determined after a single injection of estradiol or antiestrogens (Fig. 6). In the uterus of immature rat treated with vehicle, most of the receptor were found in the cytoplasm while nuclear receptor level was low. Following the injection of 50μg estradiol, the amount of nuclear estrogen receptor was sharply increased, reaching the maximum at 16 h, and then declined to the vehicle-treated level by 48 h. Nuclear receptor concentrations were reversely paralleled with cytosolic receptor which was rapidly depleted, reaching the minimum at 1 h and then sharply increased by 24 h, maintaining similar levels thereafter. However, 50μg tamoxifen or LY117018 did not alter cytosolic receptor, but increased nuclear estrogen receptor from 1 h after the injection.

Effects of estradiol and antiestrogens on progesterone receptor

By employing optimal conditions for progesterone receptor assay (Park *et al.*, 1986b; Yoon *et al.*, 1987) established in our laboratory, saturation curve and

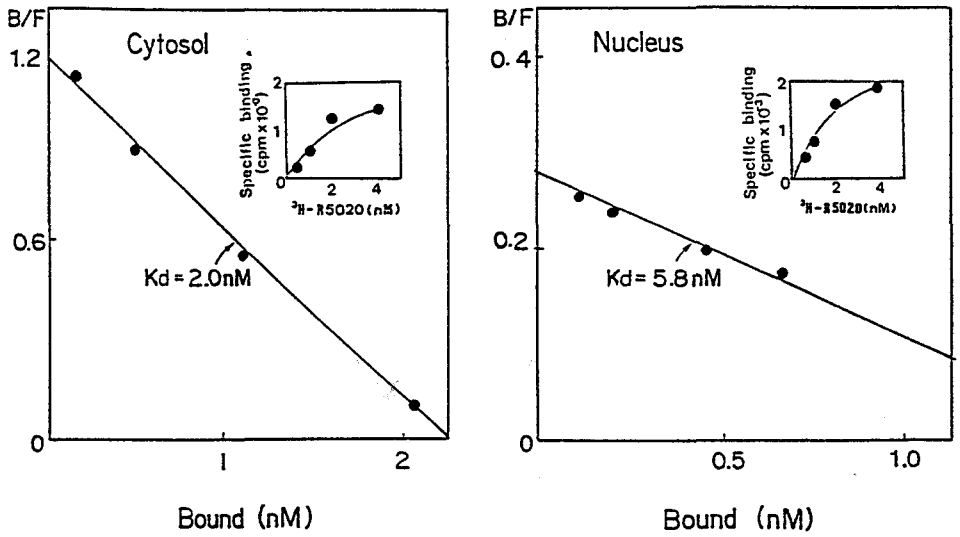

Fig. 7. Saturation curve (insert) and Scatchard analysis for progesterone receptors of immature rat uterus. Receptors were incubated with various concentrations of ^{3}H-R5020 at 4°C for 18 h.

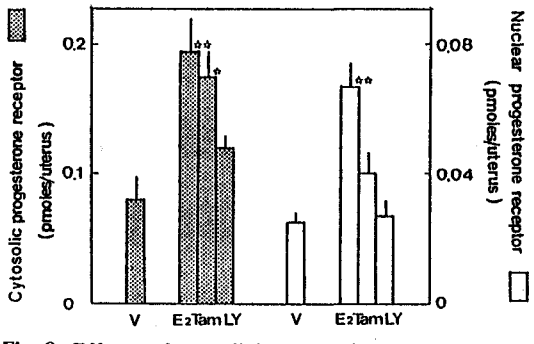

Fig. 8. Effects of estradiol and antiestrogens on progesterone receptors of immature rat uterus. Estradiol and antiestrogens were s.c. administered 100μg for 2 days. Each bar represents the mean ± SEM of 3 animals.
* $p<0.05$, ** $p<0.01$; compared with vehicle.

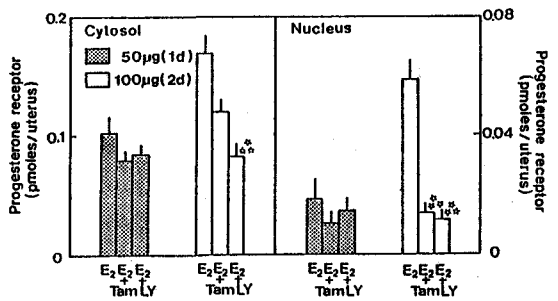

Fig. 9. Effects of antiestrogens on estrogen-induced progeserone receptors of immature rat uterus. Each bar represents the mean ± SEM of 3 animals.
*** $p<0.001$; compared with estradiol-only.

Scatchard plot were obtained (Fig. 7). Cytosolic and nuclear progesterone receptors were saturated with 4nM of ^{3}H-R5020. Kd value and binding affinity for cytosolic progesterone receptor were 2.0nM and 2.43 pmol/ml while those for nuclear progesterone receptor were 5.80nM and 1.05 pmol/ml respectively.

The effect of estradiol, tamoxifen and LY117018 on the progesterone receptor was determined by the ^{3}H-R5020 exchange assay. As shown in Fig. 8, the treatment of estradiol significantly increased both of cytosolic and nuclear progesterone receptors. Tamoxifen also significantly increased both receptors ($p<0.001$) although an increase in progesterone receptor by LY117018 was not significant.

However, the treatment of antiestrogens reduced the estradiol-induced increase in cytosolic and nuclear progesterone receptors (Fig. 9). This effect was more prominent with the treatment for two days. LY117018 significantly decreased estradiol-induced cytosolic and nuclear progesterone receptors ($p<0.01$) while tamoxifen decreased nuclear progesterone receptor ($p<0.01$).

DISCUSSION

In the present study, antiestrogens elicited partial estrogenic responses in the absence of estradiol, but in the presence of estradiol, antiestrogens antagonized the action of estradiol. The agonistic activity of tamoxifen was more effective than that of LY117018 in all parameters used in the present study. These result suggests that the estrogenic properties of antiestrogen may be due in part to a biological half-life, i.e. the length of time that antiestrogen-receptor complex is retained in the nucleus (Keeping and Lyttle, 1982; Jordan and Godsen, 1983). LY117018 has two hydroxyl groups compared with tamoxifen and the presence of two hydroxyl groups may result in a greater rate of clearance from the animal. LY117018 was superior to tamoxifen at reducing the uterotropic effects of the short acting estrogen because LY117018 is a less estrogenic than tamoxifen (Black and Goode, 1981). Uterotropic effect were greater after multiple doses of estradiol than a single dose. However with the multiple dose regimen of tamoxifen and LY117018, uterine growth was slightly increased. This inability to sustain uterine growth by repeated administration of antiestrogen may be due to the long nuclear retention time of the antagonist-receptor complex coupled with lack of cytosolic estrogen receptor replenishment (Clark *et al.*, 1973).

Antiestrogens showed less effective uterine responses with respect to increases in DNA and protein contents of uterus compared with estradiol treatment. Keeping and Lyttle (1982) reported that maximal increases in uterine weight, DNA content, and peroxidase activity did not occur until 36-60 h after a single injection of tamoxifen. Waters and Knowler (1981) have shown that after a single injection of tamoxifen to immature rats, total uterine RNA synthesis peaked at 12-32 h, while RNA synthesis peaked at 2-4 h after the administration of estradiol. We measured the uterine growth at 24 h after the administration of estradiol and antiestrogens without considering delayed uterine response to antiestrogens (Koseki *et al.*, 1977; Borgna and Rochefort, 1981). Accordingly, low agonistic effect was observed by the treatment of antiestrogens.

The large increase in uterine peroxidase activity after the treatment of immature or ovariectomized rats with estradiol has an implication that this enzyme may be a specific marker protein in tissues responsive to estrogen (Lyttle and DeSombre, 1977; Jellinck *et al.*, 1979). The present study shows that estradiol stimulated peroxidase activity, while tamoxifen and LY117018 slightly increased peroxidase activity (Keeping and Lyttle, 1982). But co-treatment of antiestrogen and estradiol significantly decreased the estradiol-stimulated increase in peroxidase activity. This finding also suggests that antiestrogens are agonistic or antagonistic on uterine peroxidase activity depending upon estrogen milieu.

Estradiol and antiestrogens are known to stimulate synthesis of uterine progesterone receptor in several species (Koseki *et al.*, 1977; Jordan and Prestwich, 1978). In our study, estradiol and tamoxifen significantly increased nuclear and cytosolic progesterone receptors although LY117018 slightly increased. This result is consistent with the previous data that LY117018 is short-acting and less agonistic with various parameters for estrogenic action. When antiestrogens were co-treated with estradiol, progesterone receptors were greatly antagonized by multiple injections than a single injection. Complete estrogen antagonism may be achieved by continuous occupation of the receptors by antiestrogen so that the tissue response is dependent upon the low intrinsic activity of the antiestrogenic ligand complex. However, the precise mechanisms underlying the action of antiestrogens are unclear (Jordan *et al.*, 1978; Katzenellenbogen *et al.*, 1979). The observations that there is a binding site(s) for antiestrogens (antiestrogen binding protein) that is insensitive to competition by estradiol (Sutherland *et al.*, 1980; Jordan and Bowser-Finn, 1982) argues for separate, and perhaps more complex mechanisms of action for the nonsteroidal antiestrogens. Indeed, Black and Goode (1981) have suggested that antiestrogens can be divided into two categories: those that function through the estrogen receptor mechanism and those that function through the antiestrogen binding protein as well as the estrogen receptor. In our data, estradiol and LY117018 bind to the estrogen receptor and form a high affinity complex, while tamoxifen showed a lower affinity to the estrogen receptor. LY117018 (77%) had a lower affinity for estrogen receptor than estradiol (100%) but had 12-fold higher affinity compared with tamoxifen. Tamoxifen (6.3%) had a very low affinity for estrogen receptor. Sudo (1983) suggested that LY117018 had a higher affinity (200%) compared with estradiol and diethylstilbestrol. This report is slightly different from our result but consistent with the fact that LY117018 had a high affinity for estrogen receptor than trans-tamoxifen.

Thus, LY117018 exhibits high affinity for estrogen receptor, yet it elicits minimal uterotropic activity.

Accordingly, LY117018 can block or antagonize the uterotropic action of estradiol by way of competitive antagonism. On the other hand, tamoxifen is itself elicits significant uterotropic activity, but it forms a low affinity complex with estrogen receptors. Studies employing ^{3}H-tamoxifen have detected an interaction of the compound with class of receptor sites that are distinct from specific estrogen receptors (Sutherland *et al.*, 1980). Black and Goode (1981) have shown that LY117018 can not block or regress uterotropic effects of tamoxifen or its hydroxylated metabolite, suggesting that these two antiestrogens might act at a separate site or by different molecular mechanisms.

Tamoxifen is known to be triphenylethylene antiestrogen with antitumor activity, but, its mechanism is not completely understood. Recently, it has been shown that tamoxifen is a potent inhibitor of eucaryotic protein synthesis in Xenopus oocytes, intact reticulocytes and reticulocyte lysate (Knowland *et al.*, 1984; Guille an Arnstein, 1986). Tamoxifen appears to act by inhibiting polypetide chain elongation. This action of tamoxifen is independent of estrogen receptor and may explain its therapeutic effectiveness in estrogen-independent tumors. Also, it is reported that tamoxifen is an antagonist of calmodulin, a major cellular calcium receptor and calcium dependent regulator of many cellular processes, and the antagonism of calmodulin by tamoxifen may be one of the mechanisms responsible for its pharmacological actions (Lam, 1984). Another report suggests that the growth-inhibitory and cytotoxic effects of tamoxifen may be in part due to its effects on protein kinase C (O'Brian *et al.*, 1985). But establishing the possible role of estrogen-insensitive binding sites in tumor induction and/or regression, as well as the significance of the differential biological influence of LY117018 and tamoxifen observed here will require further investigation.

From these results, it is therefore suggested that LY117018 is a potent antiestrogen with less estrogenic activity compared with tamoxifen and tamoxifen with a low affinity for estrogen receptor might act at a different site or by other molecular mechanisms than LY117018 which elicits the action by binding to estrogen receptor.

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= 국문초록 =

자궁세포 성장에 미치는 항에스트로젠제의 작용기전

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비스테로이드성 항에스트로젠제는 표적기관에서 estrogen 수용체와 상경적으로 결합하므로써 estrogen의 작용을 억제하는 것으로 알려져 있다. 비스테로이드성 항에스트로젠제는 대체로 triphenylethylene계로서 tamoxifen, clomiphene, LY117018등이 있으며 표적기관에서 estrogen의 작용을 억제하기 때문에 estrogen과 관련된 질환을 치료하는데 이용되어 오고 있다. 본 연구에서는 생후 21-23일된 미성숙 흰쥐를 재료로 항에스트로젠제중 tamoxifen과 LY117018이 자궁세포 성장에 어떠한 영향을 미치며 어떠한 기전으로 estrogen의 작용을 길항하는지를 규명하고자, 항에스트로젠제가 estrogen 작용의 중요 지표에 미치는 영향을 비교 관찰하여 다음과 같은 결과를 얻었다.

Tamoxifen과 LY117018은 자궁세포에서 estrogen의 영향이 없는 경우에는 estrogen agonist로, estrogen 작용하에서는 estrogen antagonist로서 작용하였다. Estrogen 작용의 여러가지 지표에 대해 tamoxifen이 LY117018보다 agonistic effect는 더 컸으나, antagonistic effect는 LY117018이 더 큰 것으로 나타났다. Estrogen 수용체에 대한 결합능은 LY117018이 estradiol 보다는 약간 낮았으나 용량에 비례하여 estrogen 수용체와 결합하였다. 그러나 tamoxifen은 estrogen 수용체에 대한 결합이 아주 낮았다. Estrogen 수용체에 대한 binding affinity는 estradiol(100%), LY117018(77%), tamoxifen (6.3%) 순으로 나타났다. 항에스트로젠제의 생체내 투여는 estrogen 존재 유무에 따라 estrogen 수용체 농도에 agonist 또는 antagonist로 작용하였다. 항에스트로젠제의 단독투여는 progesterone 수용체 생성을 증가시키나, estrogen에 의하여 유도된 progesterone 수용체 생성을 억제하였다.

이상의 결과로 보아, tamoxifen과 LY117018은 estrogen 유무에 따라 흰쥐 자궁세포에서 estrogen antagonist로서 뿐만 아니라 agonist로서도 작용함을 알 수 있다. 그러나 estrogen수용체와의 결합능력이 아주 낮은 tamoxifen은, 용량에 비례하여 estrogen 수용체에 결합하므로써 작용하는 LY117018과는 다른 기전으로 작용하는 것으로 생각된다.