The Effects of Tibolone in Breast Cancer Cell Lines- The Role of Tibolone in Phospholipase D Signal Transduction Pathway

[1]Department of Surgery, Seoul National University College of Medicine, Seoul,
[2]Cancer Research Institute, Seoul National University College of Medicine, Seoul,
[3]Department of Biochemistry, Hanyang University College of Medicine, Seoul, Korea

Ki-Wook Chung[1], Soo-Jung Ahn[2], Sung Won Kim[1], Wonshik Han[1], Hee Joung Kim[1], Ji Yeon Bae[2], Joong-Soo Han[3], and Dong-Young Noh[1,2]

Purpose: Tibolone is a tissue specific steroid hormone newly recognized as an estrogenic agent for hormone replacement therapy (HRT). The aim of the study is to characterize the basic mechanism of tibolone in the PLD signal transduction pathway of breast cancer cell lines.
Methods: The levels of phospholipase D (PLD), caspase 3 mRNA and protein, and the cell counts were measured in estrogen receptor positive MCF-7 and negative MDA-MB-231 cell lines treated with estradiol, tamoxifen and tibolone and three metabolite forms of tibolone (3β-OH-tibolone, △4 isomer, 3α-OH-tibolone). Multimodality methods such as RT-PCR, immunoblot analysis and in vivo enzyme activity assay were used.
Results: The addition of estradiol to MCF-7 cell line resulted in cell proliferation in a time-dependent manner while that of tamoxifen and tibolone showed antiproliferative effects. The addition of tamoxifen or tibolone to MCF-7 and MDA-MB-231 cell lines resulted in the elevation of caspase 3 mRNA levels, indicating the induction of apoptosis. PLD mRNA level was elevated in both cell lines treated with tamoxifen, but decreased in those treated with the various tibolones, except for 3β- OH-tibolone. In immunoblot analysis, while MCF-7 cell line treated with tamoxifen showed an increased level of PLD expression, in MDA-MB-231 cell line the expression was decreased. Similar results were observed in the addition of tibolones, which resulted in an increase of PLD expression level in MCF-7 cell line and a decrease in MDA-MB-231 cell line. In vitro PLD activity assay showed decreased activity after estradiol treatment and increased activity after tamoxifen and tibolone treatment in MDA-MB231 cell line. In MCF-7 cell line,among the tibolones only △4 isomer increased PLD activity. Tibolone-showed antiproliferative and apoptosis-inducing effects on MCF- 7 and MDA-MB-231 cell lines. But its influence on the signal transduction pathway varied slightly between the two cell lines.
Conclusion: we were able to find the antiestrogenic properties of the estrogenic agent tibolone. **(Journal of Korean Breast Cancer Society 2003;6:1-7)**

Key Words: Tibolone, Phospholipase D, Signal transduction pathway, Apoptosis, Estrogen receptor

INTRODUCTION

Hormone replacement therapy (HRT) in postmenopausal women is important in improving quality of life. HRT can help hot flush, bone pain and many other climacteric symptoms. (*1-3*) However, a possible HRT hazard in the development of breast cancer has restricted its use in breast cancer patients despite several conflicting results.(*4,5*) Although the available data is contradictory, it is generally accepted that long term or high dose estrogen treatment may lead to small increase in relative risk of breast cancer.(*6*)

Tibolone is a synthetic steroid with tissue specific estrogenic, progestogenic and androgenic activity. By virtue of being rapidly converted into three active metabolites (△4 isomer, 3α-OH-metabolites and 3β-OH-metabolites) with different specific effects on different tissues, tibolone is thought to have tissue specific activity.(*7*) It is known as an effective therapeutic agent for hot flush, bone pain, dyspareunia, and mood disorders without exerting proliferative effect in the breast and endometrium.(*8,9*) It can decrease the local production of estrogen in human breast tissues by inhibition of sulfatase,(*10*) which

Correspondence: Dong-Young Noh, Department of Surgery, Seoul National University College of Medicine, 28 Yongon-dong, Chongno-gu, Seoul 110-744 Korea (Tel) 02-760-2921, (Fax) 02-766-3975, (E-mail) dynoh@plaza.snu.ac.kr

Received: February 8, 2003 Accepted: March 20, 2003
This study was supported by a grant (no. 2000-2-20900-003-3) from the Korea Science and Engineering Foundation (KOSEF) and in part by Organon International.

suggests that tibolone can be a useful alternative hormone replacement agent in patients at risk of breast cancer. Tibolone also demonstrated an apoptosis inducing effect in breast cancer cells.(*11*) But the molecular effect of tibolone in various signal transduction pathways has not been well confirmed. The signal transduction system is a coordinate system that switches external stimuli such as growth factors, differentiation factors, hormones, neurotransmitters and light to internal second messenger and associated proliferation, carcinogenesis and apoptosis. Phospholipase D (PLD) is one of the important enzymes that function as a growth factor signal transduction pathway. Stimulation of PLD occurs in a wide variety of cells treated with hormone and growth factors.(*12-14*) PLD catalyzes the hydrolysis of phosphatidylcholine (PC) to produce phosphatidic acid (PA) and choline. PA is metabolized to diacylglycerol (DAG), a protein kinase C (PKC) activator.(*15*) In humans, an increase in PKC activity has been demonstrated in breast tumors(*16*) and Noh et al. also reported an overexpression of PLD1 in human breast cancer tissues relative to normal tissues.(*17*) These findings suggest PLD activation may enhance proliferation, differentiation and carcinogenesis. Since estrogen plays a role in the regulation of PLD (Noh et al, unpublished data), we assumed that tibolone might exert an influence on PLD signal transduction. So we analyzed PLD expression and activity after treatment of estrogen receptor bearing MCF-7 cell line and estrogen receptor not bearing MDA-MB-231 cell line with estradiol, tamoxifen, tibolone and its metabolites.

METHODS

1) Materials and cell lines

PMSF, leupeptin, aprotinin, Triton X-100, DTT, Hepes and diethylpyrocarbonate (DEPC) were obtained from Sigma Chemical Co. Trizol reagent was obtained from Gibco BRL, the First strand cDNA synthesis kit from Boehringer Mannheim, Taq DNA polymerase from TaKaRa Shuzo Co., protein G Sepharose from Pharmacia Biotech Products, peroxidase conjugated goat anti-rabbit IgG antibody from Jackson ImmunoResearch Laboratories, Inc., and the Enhanced Chemiluminescence Detection (ECL) system from Amersham Corp. The protein concentration was determined using a protein assay kit from Pierce. Two human breast cancer cell lines, MCF 7 (ER+, PR+, bcl-2+) and MDA-MB-231 (ER-, PR-), were used in this study. Estradiol and tamoxifen were purchased from standard commercial suppliers. Tibolone and its metabolites were kindly supplied by Organon international. Antibody for PLD was provided by professor Min (Department of Physiology, College of Medicine The Catholic University of Korea, Seoul, Korea).

Table 1. Primers used for RT-PCR

Markers		Sequences (5'-3')
*h*PLD1	Forward	TGGGCTCACCATGAGAA
	Reverse	GTCATGCCAGGGCATCCGGGG
Caspase 3	Forward	ATACTCCTTCCATCAACTAG
	Reverse	AACATCACAAAACCATAATC
β-actin	Forward	CACTGTGTTGGCGTACAGGT
	Reverse	TCATCACCATTGGCAATGAG

2) Cell culture

MCF 7 and MB-MDA-231 cells were maintained in medium A (RPMI 1640, 2 mM glutamine, 100 U/ml penicillin, 100 g/ml streptomycin, 5% fetal bovine serum) in 75 ml flasks (Falcon) and grown in the presence of 5% CO_2 in air at 37°C. Cells were switched into medium B (phenol red-free RPMI 1640, 2 mM glutamine, 100 U/ml penicillin, 100μg/ml streptomycin, 5% dextran coated charcoal stripped fetal bovine serum) to remove the effect of exogeneous estrogen. Each cell line was treated with estradiol, tamoxifen, tibolone and its metabolites 3αOH tibolone, 3βOH tibolone and △4 isomer. Each estradiol, tamoxifen, tibolone and its metabolites were solubilized with ethanol at a concentration of 1 nM, 1μM, and 1μM respectively. The effects of treatment were monitored at the time point 0, 3, 6, 24, 48 and 72 hours. After each period, cell count was measured under light microscopy (×40).

3) RNA isolation and RT-PCR

Each cell line was collected in eppendorf tube, and total RNA was extracted by routine Trizol® (Gibco-BRL Co) method. The RNA pellet was dissolved in diethylpyrocarbonate (DEPC)-treated H_2O to concentrations of approximately 0.5 microgram per milliliter to 1.0 microgram per milliliter and stored at -70°C. The quantity and quality of the RNA preparations were determined by absorbance at 260 nm and 280 nm. One microgram of total RNA sample was reverse transcribed using MMLV-Reverse Transcriptase with random primer $p(dN)_6$, according to the manufacturer's instructions. Produced cDNA were purified using phenol:chloroform:isoamyl alcohol 25 : 24 : 1 solution and dissolved in sterile distilled water. PCR analysis was performed as described. Primers used for amplification are listed in table 1. Amplification conditions used consisted of 30 cycles of 94°C for 30 seconds, 60°C for 1 minute, and 72°C for 1minute. PCR products were electro-

phoresed in 1.5% agarose gel and stained with ethidium bromide. Stained gels were pictured by the BIPS 3.0 system with computer image analyzer. Beta-actin was used for internal validation.

4) Immunoblot analysis

Cell lines were cultured for 2 hr prior to the addition of estrogen, tamoxifen and tibolone. After treatment, cells were scrapped in cold-lysis buffer and cytosolic and membrane fractions were prepared according to the methods of Exton. Proteins were determined using the BCA protein Assay reagent system. Equal amounts of protein were loaded on a 10% SDS-PAGE. Separated proteins were transferred to nitrocellulose paper and analyzed for the presence of PLD using proper antibodies. Goat anti-mouse antibody (1 : 1,000) and Goat anti-rabbit antibody (Jackson immunoresearch Inc., Pennsylvania USA) were used as second antibodies. Immune complexes were detected with the ECL (Enhanced Chemi Luminescence detection, Amersham, UK) method.

5) In vitro PLD enzyme activity assay

The two cell lines described above were used in this experiment to identify the tibolone effect on cell signaling. Cells in 6-well plates were labeled 1 day before confluence with [^{3}H] myristic acid for 24 hours. Cells were labeled for 3 days with [^{3}H] glycerol. For all experiments employing [^{3}H] myristic acid, cell monolayers after labeling were rinsed 2 times with [^{3}H] glycerol and equilibrated for 5 hr in phenol red free medium containing 0.3 mg BSA/ml. After one final wash, cells were exposed to tamoxifen and tibolone as indicated. After cell treatment, media were aspirated and total cellular lipids were extracted.

[^{3}H] PA, [^{3}H] DG and [^{3}H] phosphatidylethanol were resolved from total cellular lipids by TLC employing solvent systems. The lipids, identified by co-migration with commercial standards, were visualized in iodine apor. Radio-activity was determined in the silica gel scrapings by liquid scintillation spectrometry. Areas of the gel were first scraped into 0.5 ml water, then 4ml of EcoLume was added and the contents of the plastic minivials were mixed before counting.

Water-soluble phospholipid metabolites were separated into base and phosphoryl-base moieties on a Dowex 50 W acidic cation exchanger using Bio-Rad Poly-prep 10 ml columns. Phosphobases were eluted with water and free bases with 10 ml 1.0 N HCl. Samples were assayed for radioactivity by liquid scintillation spectrometry.

6) Statistical analysis

Independent T test was used to compare the means of cell number and PLD activity between different treatment groups.

RESULTS

1) Tibolone effects on cell viability and apoptosis

MCF 7 cell lines treated with estradiol, tamoxifen, and tibolone and metabolites were monitored at 3 hr, 6 hr, 24 hr, 48 hr and 72 hr after treatment for viable cell number count by light microscope. Fig. 1 shows a time dependent increase of cell numbers up to 1.5 fold compared to the control (no treatment) after estradiol treatment ($P < 0.05$). On the contrary,

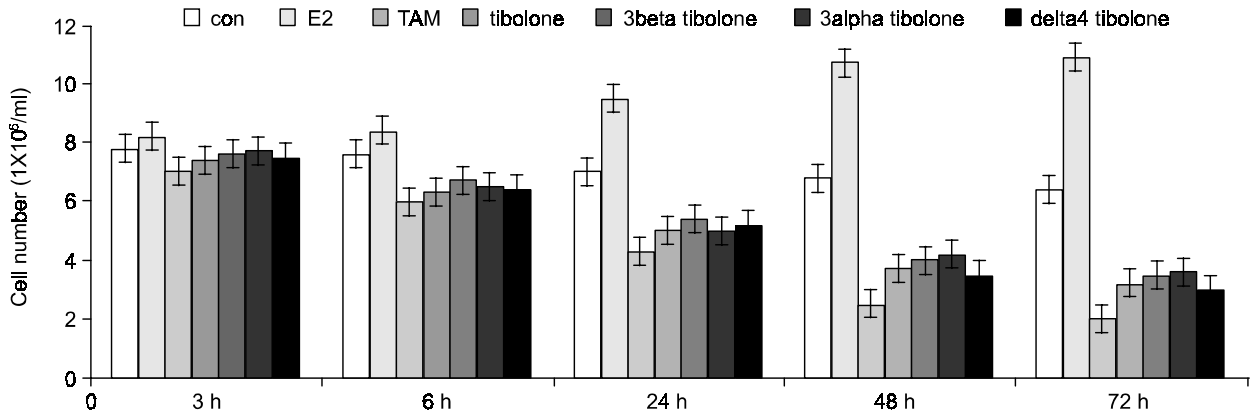

Fig. 1. Cell viability after estradiol, tamoxifen and tibolone treatment in MCF-7 cell line. Estradiol increased cell growth in MCF7 cell line compared with control while tamoxifen and tibolone decreased cell number ($P < 0.05$, respectively). (Con = control, E2= estradiol; TAM = tamoxifen).

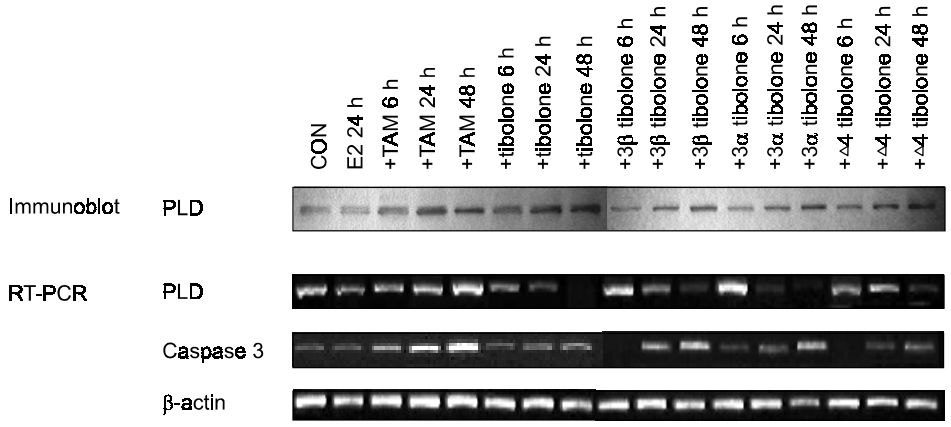

Fig. 2. RT-PCR and immunoblots for PLD and caspase 3 after estradiol, tamoxifen and tibolone treatment in MCF-7 cell line (Con = control, E2 = estradiol, TAM = tamoxifen).

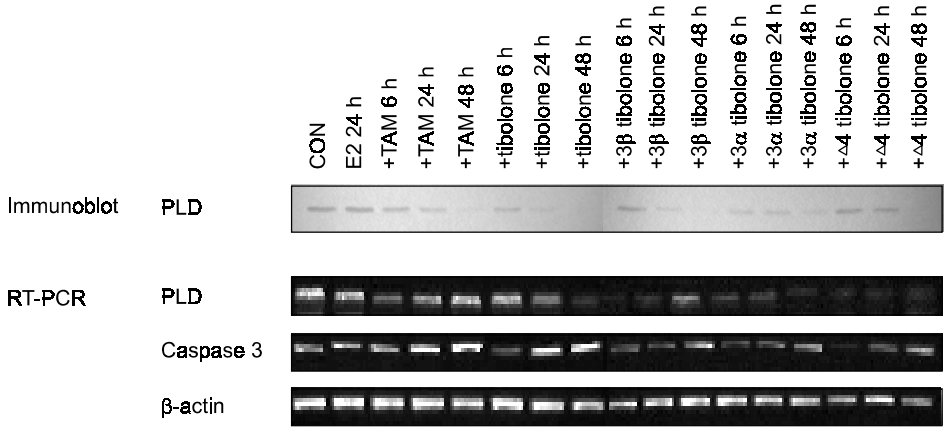

Fig. 3. RT-PCR and immunoblots for PLD and caspase 3 after estradiol, tamoxifen and tibolone treatment in MDA-MB-231 cell line (Con = control, E2 = estradiol, TAM = tamoxifen).

a serial decrease of cell numbers was observed after tamoxifen and tibolone treatment ($P < 0.05$). At 72 hrs after treatment, tamoxifen presented approximately a 75% decrease in cell numbers and tibolone a 50～60% decrease. No definite differences were evident between tibolone and its metabolites. The same results were observed in MDA-MB-231 cell line (data not shown) except in case of estradiol. Estradiol did not stimulate cell growth after treatment in case of MDA-MB-231 cell line. To identify whether the decrease of cell number was derived from apoptosis, RT-PCR for caspase 3, a mediator of apoptosis, was performed in two cell lines (Fig. 2 and 3). RT-PCR revealed a time dependent increase of caspase 3 in the groups treated with tamoxifen and tibolone, which suggested that the observed cell number decrease was generated by apoptosis.

2) The effect of Tibolone on PLD mRNA level and PLD protein expression

Time dependent analysis of PLD mRNA level and PLD expression in both cell lines was done by RT-PCR and immunoblot. In MCF-7 cell line (Fig. 2), after 24 hours of estradiol treatment, PLD mRNA level and PLD protein expression showed no significant difference from the control. In the case of tamoxifen treatment, both PLD protein expression and mRNA level were increased. In the tibolone treated group, PLD protein expression was increased time dependently. However, PLD mRNA level was decreased in all tibolone and its metabolites. In MDA-MB-231 cell line (Fig. 3), as in MCF-7 cell line, estradiol showed no influence on PLD expression after 24 hrs. Tamoxifen decreased PLD protein expression and increased PLD mRNA level time dependently; results which differed from MCF7 cell line. In tibolone treated cell lines, all tibolone and its metabolites showed a time dependent decrease of PLD protein expression. However, they showed variable effects on mRNA expression. (tibolone; 3α-OH-tibolone and △4 isomer showed a decrease while only 3β-OH-tibolone showed an increase).

3) The effect of tibolone on PLD activity

To evaluate the effect of tibolone on PLD activity, in vitro

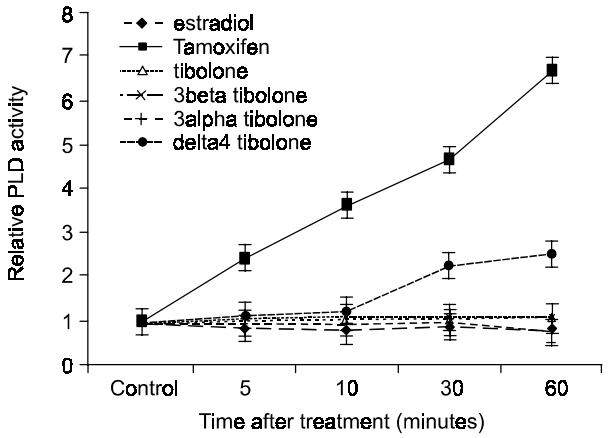

Fig. 4. In vitro PLD activity assay after estradiol, tamoxifen and tibolone treatment in MCF-7 cell line. Tamoxifen and △4 isomer showed significant increase of PLD after 60 minutes of treatment ($P<0.05$). (E2= estradiol; TAM= tam oxifen).

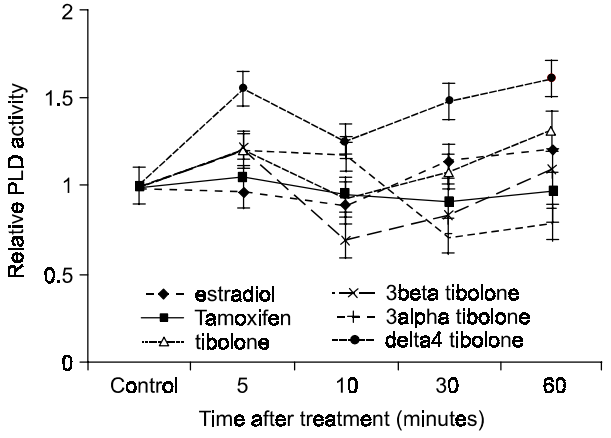

Fig. 5. In vitro PLD activity assay after estradiol, tamoxifen and tibolone treatment in MDA-MB-231 cell line. Only △4 isomer had significant increasing effect among tibolone and metabolites ($P<0.05$). (E2 = estradiol; TAM = tamoxifen).

PLD activity assay was performed. In MCF-7 cell line, estradiol slightly decreased PLD activity with a relative decrease of about 20%. But tamoxifen increased PLD activity by up to seven fold. In the case of tibolone, only △4 isomer increased PLD activity, by up to 2.5 fold ($P<0.05$), while the other metabolites did not affect PLD activity (Fig. 4). In MDA-MB-231 cell line, estradiol increased PLD activity by up to 1.2 fold after 60 minutes and tamoxifen showed only a minimal effect. In the case of tibolone, 3β-OH-tibolone and △4 isomer increased PLD activity by between 1.1 and 1.6 fold after 60 minutes, but 3α-OH-tibolone decreased the activity by about 0.8 fold after 60 minutes, although it increased the activity at 10 minutes by up to 1.2 fold (Fig. 5). In independent T test, only △4 isomer showed significant increase ($P<0.05$). As a result, the influence on PLD activity varied significantly among the estradiol, tamoxifen and tibolone treated MCF7 and MDA-MB-231 cell lines.

DISCUSSION

Tibolone is metabolized into two hydroxy forms (3αOH tibolone and 3βOH tibolone) by 3α/β hydroxysteroid dehydrogenase in the intestine and liver rapidly after administration. It also can be converted into △4 isomer by the enzyme 3β hydroxysteroid dehydrogenase-isomerase directly or after conversion to 3βOH tibolone.(*7*) Then these forms are converted into sulfated inactive forms and are activated by sulfatase.(*18*) These 3 active forms have different hormonal receptor binding affinities and this binding affinity difference is one of the explanations for the tissue specific activity (i.e. progestagenic, androgenic or estrogenic effect target tissue) of tibolone.(*19*) In the breast, many studies have reported the anti-proliferative effect of tibolone. Tibolone decreases mammographic density during administration(*8,9,20,21*) and Kloosterboer et al. reported a growth inhibition effect of tibolone in the DMBA induced mammary cancer model.(*22*) Because of this property, tibolone has been studied as a possible alternative hormonal replacement agent in postmenopausal woman. Two mechanisms had been proposed to explain this antiproliferative effect in breast tissue. First, tibolone has a breast tissue-specific sulfatase inhibitory effect(*23*) which prevents conversion to estrone from estrone sulfate(*24*) and allows estrogen deprivation to occur due to this lack of estradiol pool. The other suggested mechanism is an apoptosis inducing effect. Gompel et al. reported a decrease of bcl-2, anti apoptotic protein and DNA fragmentation in breast epithelial cells after tibolone treatment.(*11*) However, it is not clear how the apoptotic signal is transduced by tibolone.

Apoptosis is a very complicated reaction regulated via numerous signals. In tamoxifen-induced apoptosis, many findings have suggested that tamoxifen induced apoptosis signals are transferred through non-receptor mediated pathways.(*25*)

PLD also plays a role in apoptosis. Ceramide induces apoptosis via a mitochondria-caspase pathway. Several reports have shown decreased PLD activity and mRNA level in cell lines that were treated with ceramide.(*26-28*) This apoptotic signal may be transduced through protein kinase C (PKC), the main regulator of PLD.(*29*) In our experiment, tibolone showed an apoptosis inducing effect in both estrogen receptor positive

and negative cell lines, which suggests that apoptosis induced by tibolone may be mediated by some signal transduction pathway like tamoxifen. However, the effect of tibolone on PLD expression was somewhat variable. In estrogen unresponsive MDA-MB-231 cell line, PLD protein expression was decreased time dependently and caspase3 mRNA level was increased when tamoxifen and tibolone were introduced. However, the PLD mRNA level varied according to each tibolone compound. In estrogen responsive MCF7 cell line, tamoxifen and all tibolone compounds resulted in a time dependent increase of PLD protein expression despite the variable change of PLD mRNA level. So we can hypothesize, that in receptor positive cell lines, it does not seem that tibolone and tamoxifen induced apoptosis is transduced through a PLD associated signal pathway. On the contrary, in receptor negative cell line, the apoptosis signal induced by tamoxifen and tibolone may be transduced through a PLD associated signal transduction pathway. If this hypothesis is true, tibolone influences PLD expression according to receptor status and this may be due to the estrogen receptor binding capacity of tibolone.(*19*) That is, if the cells have hormonal receptors, then tibolone may increase PLD expression through the estrogen receptor, whereas if they don't, tibolone may decrease PLD expression by a receptor independent mechanism. The effect of tibolone on PLD activity was also different between the two cell lines. In MCF7 cell line, estradiol did not affect PLD activity while tamoxifen increased PLD activity by about 7 fold. On the contrary, tibolone showed no activity increase except △4 isomer. In MDA-MB-231 cell line, estradiol increased PLD activity while tamoxifen did not. All metabolite forms of tibolone increased PLD activity but only △4 isomer statistically significant increase. However, the meaning of these results is not clear.

Tibolone exhibits a unique biological property; namely its tissue specificity. This tissue specific property raises the possible use of tibolone as an alternative treatment in hormone replacement therapy for breast cancer risk patients. However, further clinical and biological studies are required for confirmation.

CONCLUSION

Tibolone demonstrated an apoptosis inducing effect in both hormonal receptor positive and negative breast cancer cell lines. Furthermore, tibolone affected the PLD associated signal transduction pathway in various ways according to the hormonal receptor status. Because these influences on the PLD associated signal transduction pathway were highly variable, elucidation of the exact mechanism of tibolone action on the PLD associated signal transduction pathway will require further research.

REFERENCES

1) Kooistra T, Emeis JJ. Hormone replacement therapy. Curr Opinion in Chem Biol 1999;3:495-9.
2 Barrett-Connor E. Hormone replacement therapy. Br Med J 1998;17:457-61.
3) Ettinger B. Overview of estrogen replacement therapy: a historical perspective. Proc Soc Exp Biol Med 1998:19:55-72.
4) Kaufman DW, Palmer JR, de Mouzon J, Rosenberg L, Stolley PD, Warshauer ME, et al. Estrogen replacement therapy and the risk of breast cancer: Results from the casecontrol surveillance study. Am J Epidemiol 1991;134: 1375-85.
5) Yang CP, Daling JR, Band PR. Non-contraceptive hormone use and risk of breast cancer. Cancer Causes Control 1992; 3:475-9.
6) Collaborative Group on Hormonal Factors in Breast Cancer: Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. Lancet 1997;350:1047-59.
7) Kloosterboer HJ. Tibolone: a steroid with a tissue-specific mode of action. J Steroid Biochem Mol Biol 2001;76:231-8.
8) Albertazzi P, Di Micco R, Zanardi E. Tibolone: a review. Maturitas 1998;30:295-305.
9) Ross LA, Alder EM. Tibolone and climacteric symptoms. Maturitas 1995;2:127-36.
10) Chetrite GS, Kloosterboer HJ, Philippe JC, Pasqualini JR. Effect of Org OD14 (Livial) and its metabolites on human estrogen sulphotransferase activity in the hormone-dependent MCF-7 and T-47D, and the hormone-independent MDA-MB-231, breast cancer cell lines. Anticancer Res 1999;19: 269-75.
11) Gompel A, Kandouz M, Siromachkova M, Lombet A, Thevenin D, Mimoun M. The effect of tibolone on proliferation, differentiation and apoptosis in normal breast cells. Gynecol Endocrinol 1997;11(suppl 1):77-9.
12) Jones GA, Carpenter G. The regulation of phospholipase C gamma 1 by phosphatidic acid. Assessment of kinetic parameters: J Biol Chem 1993;268:20845-50.
13) Exton JH. Phosphatidylcholine breakdown and signal transduction. Biochem Biophys Acta 1994;1212:26-42.
14) Lee YH, Kim HS, Pai JK, Ryu SH, Suh PG. Activation of phospholipase D induced by platelet-derived growth factor is dependent upon the level of phospholipase C-gamma 1. J Biol Chem 1994;269:26842-7.
15) Billah MM, Eckel S, Mullmann TJ, Egan RE, Siegel MI. Phosphatidylcholine hydrolysis by phospholipase D determines

phosphatidate and diglyceride levels in chemotactic peptide-stimulated human neutrophils. Involvement of phosphatidate-phosphohydrolase in signal transduction. J Biol Chem 1989; 264:17069-77.
16) Bilah MM, Anthes JC. The regulation and cellular functions of phosphatidylcholine hydrolysis. Biochem J 1990;269:281-91.
17) Noh DY, Ahn SJ, Lee RA, Seo PK. Overexpression of phospholipase D in human breast cancer tissues. Cancer Letters 2000;161:207-14.
18) Delbressin L, de Gooyer M, Kloosterboer HJ. Sulphated metabolites of tibolone show sulphatase inhibiting activity. Abstract 1639 in Endocrine Society Meeting. Toronto 2000.
19) Schoonen WGEJ, Deckers G, de Gooyer ME, de Ries R, Kloosterboer HJ. Hormonal properties of norethisteron, 7-methyl-norethisterone and their derivatives, J Steroid Biochem Mol Biol 2000;74:213-22.
20) Colacurci N, Fornaro F, De Franciscis P, Palermo M, del Vecchio W. Effects of different types of hormone replacement therapy on mammographic density. Maturitas 2000;40:159-64.
21) Colacruci N, Mele D, de Francis P, Costa V, Fortunato N, de Seta L. Effects of tibolone on the breast. Eur J Obstet Gynecol 1998;80:235-8.
22) Kloosterboer HJ, Schoonen WGEJ, Deckers G, Klijn JGM. Effect of progestagen and OrgOD14 in in vitro and in vivo tumor models. J Steroid Biochem Mol Biol 1994;49:311-18.
23) Cherite G, Kloosterboer HJ, Pasquallini JR. Effect of tibolone (OrgOD14) and its metabolites on estrone sulfatase activity in MCF-7 and T-47D mammary cancer cells. Anticancer Res 1997;17:135-40.
24) Duncan LJ, Purohit A, Howarth NM, Potter BVL, Reed MJ. Inhibition of estrone sulfatase activity by estrone-3-0-methyl-thiophosphonate: a potential therapeutic agent in breast cancer. Cancer Res 1993;19:261-8.
25) Mandlekar S, Kong ANT. Mechanisms of tamoxifen-induced apoptosis. Apoptosis 2001;6:469-77.
26) Gomez-Munoza A, Martin A, O'Brien L, Brindley N. Cell membrane permeable ceramides inhibit the stimulation of DNA synthesis and phospholipase D activity by phosphatidate and lysophosphatidate in rat fibroblast. J Biol Chem 1994; 269:8937-43.
27) Nakamura Y, Nakashima S, Ojio K, Banno Y, Miyata H, Nozawa Y. C2-ceramide inhibits IgE-mediated phospholipase D but not C activation in rat basophilic leukemia (RBL-2H3) cells. J Immunol 1996;156:256-62.
28) Venable M, Bielawaka A, Obeid LM. Ceramide inhibits phospholipase D in cell free system. J Biol Chem 1996;271: 24800-5.
29) Lavie Y, Zhang ZC, Cao HT, Han TY, Jones RC, Liu YY, et al. Tamoxifen induces selective membrane association of protein kinase C epsilon in MCF-7 human breast cancer cells. Int J Cancer 1998;77:928-32.