Biochimica et Biophysica Acta, 1153 (1993) 308–314

BBAMEM 76165

The active metabolite hydroxytamoxifen of the anticancer drug tamoxifen induces structural changes in membranes

José B.A. Custódio [a,b,*], Leonor M. Almeida [a,b] and Vítor M.C. Madeira [b]

[a] *Laboratório de Bioquímica, Faculdade de Farmácia, Universidade de Coimbra, Couraça dos Apóstolos, 51, r/c, 3000 Coimbra (Portugal) and* [b] *Centro de Neurociências, Universidade de Coimbra, 3049 Coimbra (Portugal)*

(Received 24 May 1993)

Key words: Hydroxytamoxifen; Anti-cancer drug; Antiestrogen; Breast cancer; Membrane–lipid interaction; Membrane fluidity; Fluorescent probe

The effects of hydroxytamoxifen (OHTAM) on lipid organization of pure phospholipid liposomes, native sarcoplasmic reticulum (SR) membranes and liposomes of SR lipids were evaluated by intramolecular excimer formation of 1,3-di(1-pyrenyl)propane (Py(3)Py) and by fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH) and its derivative 3-[*p*-(6-phenyl)-1,3,5-hexatrienyl]phenylpropionic acid (DPH-PA). OHTAM promotes alterations in the thermotropic profiles of DMPC, DPPC and DSPC. As detected by Py(3)Py and DPH-PA, OHTAM induces an ordering effect in the fluid phase and a fluidizing effect in the temperature range of the cooperative phase transition. In the gel phase, no significant effects are noticed, except for DSPC bilayers, where Py(3)Py and DPH-PA detect a disordering effect. In the hydrophobic region of the above membrane systems probed by DPH, OHTAM induces only a slight fluidizing effect in the range of the phase transition and a small ordering effect in the fluid phase. As detected by all probes, the drug broadens the transition profile of DMPC and shifts the main transition temperature to lower values. However, these effects, and so those observed for the fluid phase, decrease as the fatty acyl chain length increases. Moreover, the drug removes the pre-transitions of DPPC and DSPC bilayers, as probed by Py(3)Py. In fluid SR native membranes and liposomes of SR lipids, OHTAM induces a moderate ordering effect in the outer regions of the lipid bilayer, as monitored by Py(3)Py and by DPH-PA, DPH failing to detect any apparent effect, as observed for the fluid phase of pure phospholipids. Apparently, OHTAM distributes preferentially in the outer region of the lipid bilayer, without significant effect in the bulk lipid organization of the bilayer interior. The changes of OHTAM in the bilayer dynamic properties and the different location across the bilayer thickness relative to its drug promoter (Custódio et al. (1993) Biochim. Biophys. Acta 1150, 123–129) may be involved in the cytostatic activity of tamoxifen.

Introduction

Estrogens presumably act as promoters in the growth and pathogenesis of estrogen receptor (ER)-positive breast cancers, suggesting that the risk of this disease can be controlled by prophylactic antiestrogen therapy [1].

Tamoxifen (TAM) is the most widely prescribed antiestrogen for chemotherapy of breast cancer [2]. The established efficiency and low incidence of side effects have allowed the use of tamoxifen as a chemopreventive drug [3]. However, the multiple and distinct cellular effects, concerning either ER, the antiestrogen binding sites [4], prostaglandin synthetase [5], Ca^{2+}-calmodulin-dependent enzymes [6,7], protein kinase C (PKC) [8] or other proteins, remain unclear and are not accounted for by the classical ER model, suggesting that other mechanisms may be involved in its antiestrogen action.

Recently, we reported that TAM strongly incorporates into biomembranes [9], interacts with lipids and affects the bilayer physical properties [10], suggesting that the lipid-mediated physical effects may contribute to its mechanisms of action.

In humans, TAM is metabolized to several compounds which have been shown to contribute to the antiestrogenic and anticancer activity of the drug [11,12]. TAM acts in vivo indirectly via hydroxylated metabolites [13] and, among these, 4-hydroxytamoxifen (OHTAM, Fig. 1) attracted considerable interest, ow-

* Corresponding author. Fax: +351 39 27126.

Abbreviations: OHTAM, 4-hydroxytamoxifen; TAM, tamoxifen; SR, sarcoplasmic reticulum; ER, estrogen receptor; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; DSPC, distearoylphosphatidylcholine; Py(3)Py, 1,3-di(1-pyrenyl)propane; DPH-PA, 3-[*p*-(6-phenyl)-1,3,5-hexatrienyl]phenylpropionic acid; DPH, 1,6-diphenyl-1,3,5-hexatriene; DTT, dithiothreitol; PMSF, phenylmethanesulfonyl fluoride; PKC, protein kinase C.

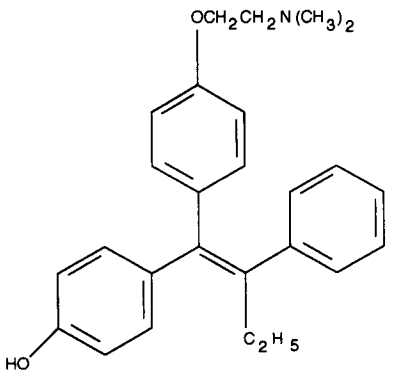

Fig. 1. Chemical structure of 4-hydroxytamoxifen.

ing to its antiestrogenic potency and higher affinity to ER than TAM [14], antagonizing the binding of the estradiol to the ER [15]. Moreover, OHTAM partitions into biomembranes [9], inhibits microsomal lipid peroxidation [16] and inhibits membrane-regulated enzymes involved in cellular proliferation [8] to a greater extent than TAM. Its high hydrophobicity suggests that the site of action of OHTAM may be the biological membrane by primarily interacting with lipids and affecting the bilayer physical properties, as we have recently shown for TAM [10].

Thus, the aim of the present work, following the previous study with TAM [10], is to elucidate the OHTAM-membrane interactions and the potential relationship between drug effects on lipid organization and its therapeutic activity. Experiments were performed on pure phospholipids, sarcoplasmic reticulum (SR) membranes (used as a native model membrane) and SR liposomes, and the effects of OHTAM on lipid organization were investigated by intramolecular excimer formation of Py(3)Py and fluorescence polarization of DPH and its analog DPH-PA, i.e., fluorescent probes that report similar physical parameters of different regions of the bilayer [17,18]. The effects are compared with those already reported for TAM [10].

Materials and Methods

Dimyristoyl-, dipalmitoyl- and distearoylphosphatidylcholine, Tris-maleate, cholesterol and DPH were purchased from Sigma. DPH-PA was from Molecular Probes (Junction City, OR), and Py(3)Py was a gift of Dr. Zachariasse from Max-Planck-Institut für Biophysikalische Chemie (Göttingen, Germany). (*Z*)-4-Hydroxytamoxifen was obtained from Amersham.

Sarcoplasmic reticulum (SR) membranes were prepared from rabbit white muscles as described elsewhere [19], except that isolation and resuspension media contained 2.5 mM DTT and about 10 μM of the protease inhibitor, PMSF. Protein was determined by the biuret method [20] using bovine serum albumin as standard. Lipids of SR were extracted as previously described [19] and the lipid contents were determined by measuring the amount of inorganic phosphate [21] after hydrolysis at 180°C in 70% $HClO_4$ [22]. Liposomes were prepared, after solvent evaporation to dryness, by dispersion in 50 mM KCl, 10 mM Tris-maleate (pH 7), and the lipid suspensions were obtained as described elsewhere [23].

Fluorescent probes of membrane fluidity were incorporated into liposomes and SR native membranes (345 μM in phospholipid) and mixtures incubated as previously described [24,25]. The molar ratios of lipid/Py(3)Py and lipid/DPH or DPH-PA were about 900 and 400, respectively. After incubation of membrane systems with the probes, OHTAM was added and the mixtures were allowed to equilibrate for 20 min at the phase transition temperature of the synthetic phospholipids and at 37°C for liposomes and native membranes of SR, before fluorescence measurements.

The fluorimetric measurements were carried out in a Perkin-Elmer LS-50 computer-controlled luminescence spectrometer provided with a thermostated cuvette. For studies with Py(3)Py, the intramolecular excimer formation was evaluated as the excimer to monomer fluorescence intensity ratio (I'/I), as previously described [24], except that the excitation and emission bandwidths were 3 nm and 4 nm, respectively. In studies with DPH and DPH-PA, the degree of fluorescence polarization (P) was determined as previously described [17], using 3 nm excitation and 4 nm emission bandwidths. Adequate control experiments were carried out without added probes to correct for the contribution of scattered light and/or background fluorescence of sample and/or solvents. Neither the solvents of drug and probes nor hydroxytamoxifen exhibit by themselves any measurable fluorescence, under our experimental conditions.

Although experiments performed in solvents at 25°C (ethanol and liquid paraffin) have shown that OHTAM induces quenching of the fluorescence of the probes, no spectral changes (neither in shape nor in the wavelength of the peak of the probes' emission spectrum) are observed, suggesting that no conformational alterations are induced in the excited state. Additionally, OHTAM does not affect either the excimer/monomer fluorescence intensity ratio (I'/I) of Py(3)Py or the fluorescence polarization of DPH and DPH-PA (unpublished data).

Results and Discussion

Effect of OHTAM in synthetic phospholipid bilayers

The interactions of OHTAM with lipid bilayers of model membranes were evaluated by three fluorescent probes differing in molecular shapes and, conse-

quently, in the positions and movements within the lipid bilayer. The selected probes were Py(3)Py, DPH and its derivative DPH-PA, since they report similar physical parameters and their locations across the membrane have been well characterized [24,26–29]. Thus, DPH polarization, reporting the rotational diffusion of the probe strongly dependent on fluidity [30,31], was used to evaluate the structural order of the lipid bilayer core. On the other hand, the intramolecular excimer formation of Py(3)Py, as reflected by the excimer/monomer ratio of fluorescence intensity (I'/I), which also depends on the fluidity of the probe environment and is determined by the rate of motion about the σ bonds of propane linking the two pyrene rings, was chosen to probe the fluidity of the outer regions of the bilayer where it preferentially distributes [24,27,28]. DPH-PA also reports the structural order of the outer regions of the bilayer, since its charged group positions at the membrane surface in contact with the water phase [18]. This probe is much less sensitive to fluorescence quenching by OHTAM than is Py(3)Py.

The term fluidity is used here in an operational sense in as far as it is reflected by (and very roughly proportional to) the rate of excimer formation by Py(3)Py or the fluorescence depolarization of DPH probes. This fluidity is related to but not identical with the physical definition of fluidity that applies to isotropic probe motion in an isotropic liquid.

The effects of OHTAM concentration in DMPC, DPPC and DSPC liposomes, as a function of temperature, evaluated by Py(3)Py and DPH-PA are shown in Fig. 2 and Fig. 3, respectively. As detected by Py(3)Py, OHTAM induces ordering and fluidizing effects dependent on temperature, i.e., a moderate decrease of I'/I in the fluid phase, reflecting a decrease of the bilayer fluidity, and a fluidizing effect is noticed in the range of the cooperative phase transition. For temperatures below the range of the phase transition, no significant effects are noticed, except as for DSPC, where Py(3)Py detects a small disordering effect promoted by OHTAM in the gel phase. The fluidizing effects of OHTAM are less apparent in liposomes of long chain (DSPC) as compared with short chain lipids (DMPC).

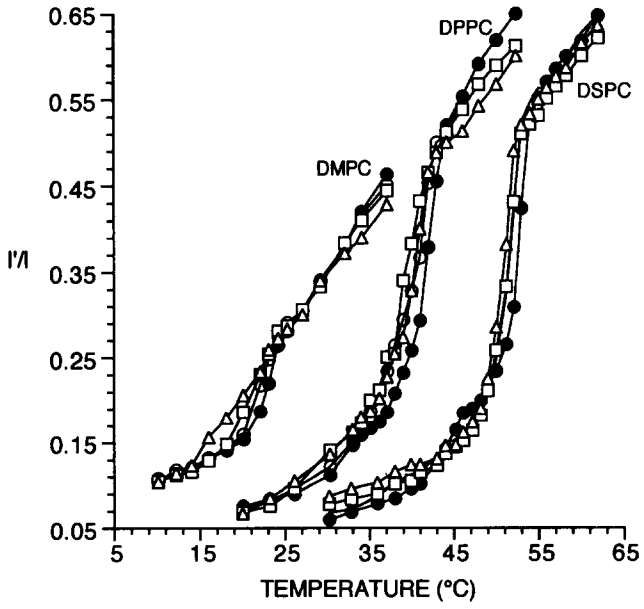

Fig. 2. Excimer/monomer fluorescence intensity ratio (I'/I) of Py(3)Py in liposomes of DMPC, DPPC and DSPC, in the absence (●) and presence of 10 (○), 25 (□) and 50 μM (△) OHTAM, as a function of temperature.

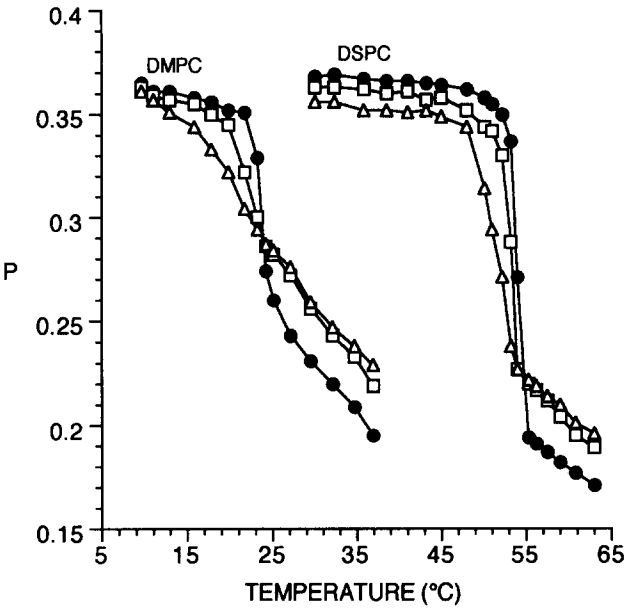

Fig. 3. Effect of OHTAM concentration on DMPC and DSPC thermotropic phase transition profiles monitored by fluorescence polarization (P) of DPH-PA as a function of temperature. (●) 0 μM, (□) 25 μM and (△) 50 μM.

The propionic acid derivative of DPH, DPH-PA, a probe apparently displaced to the surface of the membrane owing to the charge of the propionate [18], detects similar effects to those reported by Py(3)Py, as shown in Fig. 3. Therefore, DPH-PA reports an ordering effect induced by OHTAM in the fluid phase and a fluidizing effect in the temperature range of the cooperative phase transition, which it broadened, without significant effects in the gel phase of DMPC, and a small disordering effect in the gel phase of DSPC liposomes.

In principle, changes in polarization and excimer formation could also result from changes in the lifetime of probe excited states. However, effects of OHTAM on the lifetimes of these probes are probably negligible, since DPH-PA polarization and excimer formation of Py(3)Py are practically insensitive to OHTAM in the gel phase of DMPC bilayers (Figs. 2 and 3).

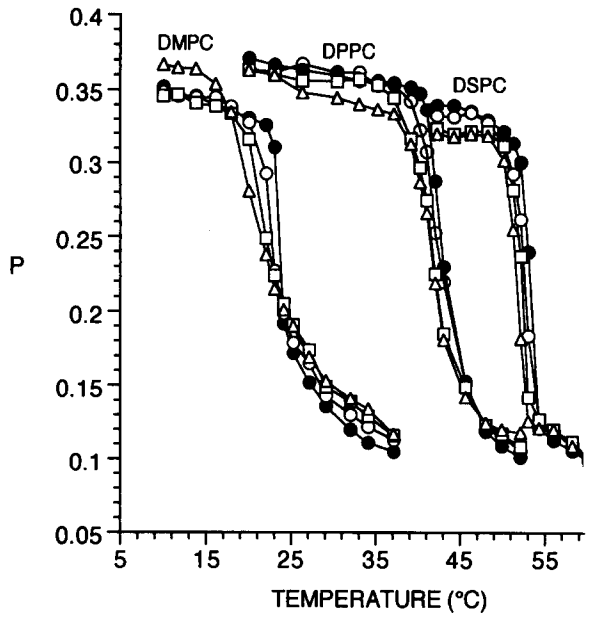

Fig. 4. Thermotropic transition profiles of DMPC, DPPC and DSPC bilayers evaluated by fluorescence polarization (P) of DPH, in the absence (●) and presence of OHTAM: 10 (○), 25 (□) and 50 μM (△).

The effects of OHTAM on the three membrane systems, as monitored by fluorescence polarization of DPH (Fig. 4), a probe displaced to the hydrophobic bilayer center [17], are smaller as compared with those probed by DPH-PA. In fact, DPH monitors only a slight fluidity alteration promoted by OHTAM, i.e., a moderate fluidizing effect in the temperature range of the phase transition and a small condensing effect in the fluid phase. Conversely, tamoxifen, locating preferentially in the hydrophobic core of membrane systems, induces strong fluidizing effects in the gel phase as detected by DPH polarization [10].

The most evident effects on lipid bilayer organization induced by OHTAM are detected over the temperature range of the main phase transition, at which the drug incorporation is maximal [9]. As monitored by Py(3)Py and DPH-PA, the drug broadens the transition profile of DMPC and shifts to lower values the main transition temperature (Figs. 2 and 3), suggesting, according to Jain and Wu [32], a preferential localization of OHTAM in the cooperativity region of the DMPC bilayer, i.e., aligning with the segment corresponding to the first eight carbons of the acyl chains. However, DPH putatively located in the bilayer center also detects a shift of the main phase transition but a less pronounced broadening of the transition profile as compared with the other probes. In the case of DMPC and as a consequence of the shorter aliphatic chains, the perturbations in the cooperative region are likely to extend to the bilayer center. The extent of OHTAM fluidizing effect on the transition phase temperature is less apparent for long chain phospholipid species (DPPC and DSPC), as detected by all fluidity probes.

In addition to the main transition, pre-transitions of DPPC and DSPC are also detected by Py(3)Py, in terms of the I'/I ratio (Fig. 2), as expected, considering that the probe preferentially distributes in the outer regions of the bilayer [17]. OHTAM, even at the lowest added concentrations, removes DPPC and DSPC pre-transitions, an effect similar to that of other molecules that apparently align with the aliphatic chains of membrane lipids close to the cooperativity region [33,34]. At the same drug concentration in the lipid suspension, this effect is more clear for OHTAM than for TAM [10].

Although OHTAM and TAM shift and broaden the main phase transition profile of DMPC in a similar way, the overall effects of OHTAM on the thermotropic behaviour of DPPC and DSPC bilayers detected by Py(3)Py and DPH are significantly smaller than those induced by TAM [10]. Therefore, the shift of the mid-transition temperature does not correlate with the antiestrogenic potency of these drugs, since the most potent OHTAM [14] is the less efficient in promoting thermotropic shifts, although its partition exceeds that of TAM [9]. In contrast with these effects in the main phase transition, the induced perturbations in the bilayer pre-transitions correlate better with the drug antiestrogenic potency, suggesting, as proposed for anesthetics [35], that pre-transition perturbations may better indicate drug-lipid interactions and clinical potency.

The concentrations of OHTAM used in this study reasonably compare with the concentrations used in other reported works [8,16]. However, it is difficult precisely to calculate the concentrations of OHTAM resulting from TAM pharmacological doses, since TAM undergoes extensive metabolic transformation and the recommended daily dose, depending upon country, is 10 to 20 mg and the range of serum levels is relatively large (50–300 ng/ml) [2]. Moreover, OHTAM, owing to its highly hydrophobic character, partitions strongly into biological membranes [9] and it is retained on receptors in various target tissues due to its high affinity [13]. Therefore, its apparent distribution volume is high and the levels of TAM and its metabolites, in humans, are 10- to 60-fold higher in tissues than in serum [36]. Thus, the estimated concentrations of OHTAM in peripheral tissues may reach values within the range of our studies.

OHTAM effects in bilayers enriched with cholesterol

Previously, it has been shown that alterations in the physical properties of membranes induced by temperature and cholesterol modify the partition of OHTAM [9]. Moreover, considering that cholesterol modulates the effects of membrane active drugs [37] and that

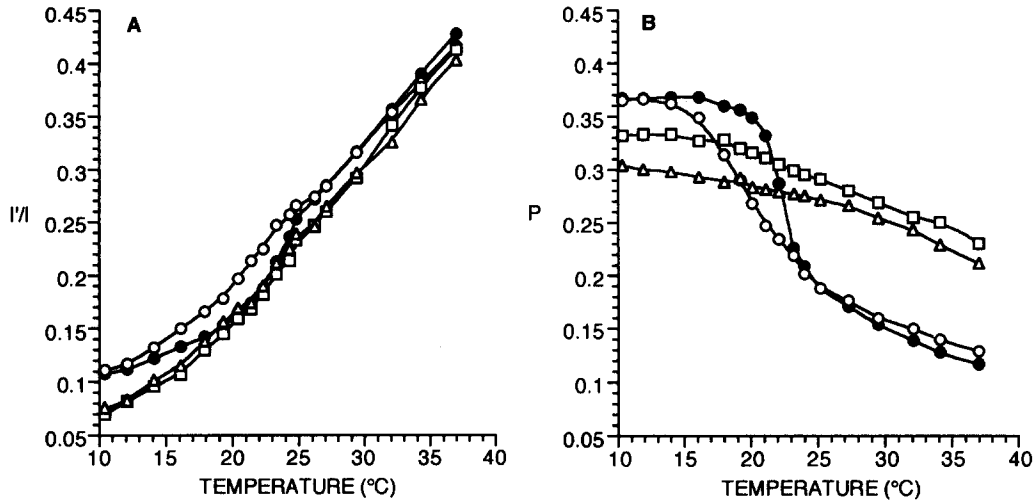

Fig. 5. Effect of OHTAM on DMPC-cholesterol bilayer organization as a function of temperature, determined by Py(3)Py I'/I ratio (A) and fluorescence polarization (P) of DPH. (●) DMPC alone, (○) 50 μM OHTAM, (□) 20 mol% cholesterol and (△) 20 mol% cholesterol plus 50 μM OHTAM.

plasma membranes of tumor cells have been shown to possess altered lipid fluidity [38], the effects of OHTAM on the fluidity of lipid bilayers enriched with cholesterol were also studied as a function of temperature (Fig. 5).

In DMPC bilayers enriched with cholesterol, OHTAM does not induce any apparent effect on the thermotropic profile, as detected by Py(3)Py (Fig. 5A), in agreement with previous partition studies showing that cholesterol incorporation drastically decreases the partitioning of OHTAM into DMPC bilayers [9]. Thus, the effects of 50 μM OHTAM in DMPC bilayers containing 20 mol% cholesterol are almost negligible as compared with those observed in the absence of the sterol. This capacity of the sterol to inhibit OHTAM effects in DMPC bilayers is stronger than that observed for TAM [10], probably as a consequence of OHTAM distribution in domains of the membrane where cholesterol is located. However, the cholesterol molecule located in the outer cooperativity region of the bilayer, with its steroid ring extending to the depth of 7–10th carbon atoms of lipid chains [39], affects strongly the cooperativity region of the bilayer evaluated either by excimer formation of Py(3)Py or by DPH polarization as compared with OHTAM (Fig. 5). In fact, the antiestrogen induces a lesser broadening on the thermotropic transition profile than cholesterol as detected by DPH (Fig. 5B). In agreement with the effects observed in more ordered lipid bilayers, OHTAM putatively displaced towards the polar region

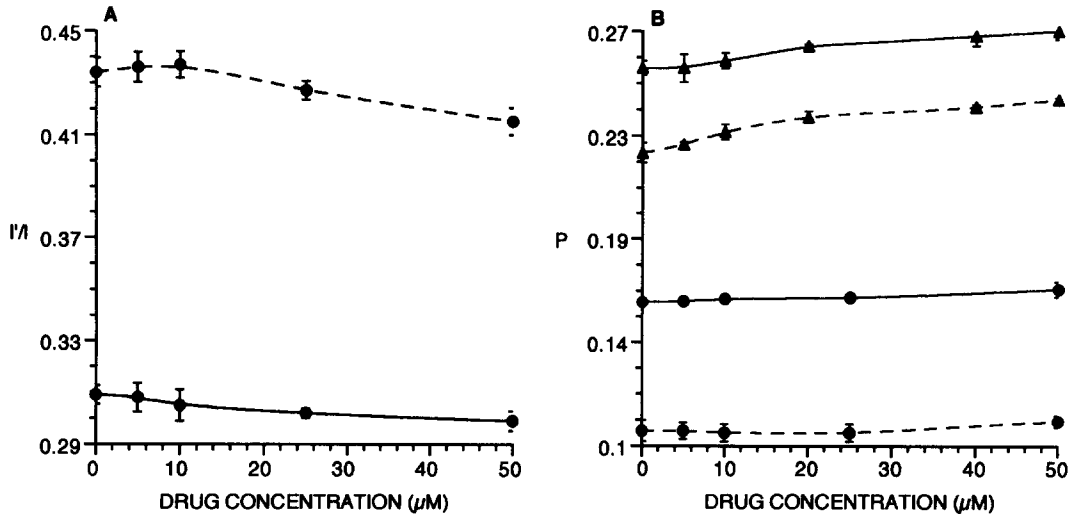

Fig. 6. Effect of OHTAM concentration, at 37°C, in native SR membranes (solid lines) and liposomes of SR lipids (hatched lines), evaluated by intramolecular excimer formation of Py(3)Py (I'/I) (A) and fluorescence polarization (P) of DPH (●) and DPH-PA (▲) (B). Data are means ± S.D. for three experiments. Standard error bars are generally encompassed within the size of the symbols.

of the bilayer induces a smaller perturbation of the cooperativity region as compared with cholesterol.

In the hydrophobic core probed by DPH (Fig. 5B), OHTAM exerts a stronger fluidizing effect than TAM in the range of gel phase temperatures in the presence of cholesterol and partially inhibits the sterol ordering action in the range of liquid-crystalline phase temperatures. These effects suggest that OHTAM, in membranes of altered lipid fluidity, such as the plasma membrane of tumor cells, may modulate the degree of order of the bilayer, thereby possibly contributing to the control of important membrane functions, including the mechanisms involved in the inhibition of cell proliferation.

Interactions of OHTAM with native membranes and liposomes of SR

The effects of OHTAM concentration, in the range of 5 to 50 μM, in native SR membranes and in liposomes of SR lipids at 37°C were investigated by Py(3)Py I'/I ratio, DPH and DPH-PA polarization (Fig. 6).

The studies with DPH do not detect any significant effect of OHTAM on the lipid organization of fluid SR native membranes or SR lipid liposomes (Fig. 6B), in agreement with the observed effects in the liquid-crystalline phase of pure phospholipids (Fig. 4), suggesting that OHTAM in fluid phase does not affect the bulk structure of the hydrophobic core of the lipid bilayer where DPH is located.

In the outer region of the lipid bilayer, OHTAM induces a small concentration-dependent ordering effect in fluid native membranes and liposomes of SR lipids as indicated by a small decrease of Py(3)Py I'/I ratio (Fig. 6A). These effects are better detected by DPH-PA in native membranes and SR liposomes (Fig. 6B). Therefore, the drug in native membranes does not extensively disturb interactions between the fatty acyl chains of the bulk bilayer phospholipids. However, the drug ordering effects are more pronounced in SR lipid liposomes, as expected and according to the effects in pure phospholipids, since the native membranes are more ordered than liposomes of their lipid extracts (Fig. 6A). Moreover, in native SR membranes, the antiestrogen may strongly bind to membrane proteins, in agreement with its high efficiency to bind to proteins in a non-specific manner [40].

The inhibition of the activity of PKC, an enzyme which is thought to be involved in cell proliferation, by triphenylethylene antiestrogens appears to be due to interactions with phospholipids rather than to direct interactions with the active site of the enzyme, as shown by O'Brian et al. [8]. Moreover, these drugs are also potent inhibitors of (Ca^{2+},Mg^{2+})-ATPase of red blood cell membranes [41] and SR membranes (unpublished data). Although, recent evidence suggests that only a minority of membrane bound enzymes is regulated by membrane fluidity [42], it is known that the Ca^{2+}-pump of SR membranes is very sensitive to changes at the interface membrane lipids. On the other hand, OHTAM is a more potent inhibitor of PKC [8] and Ca^{2+}-ATPase of SR membranes (unpublished data) than TAM and affects preferentially the polar region of the bilayer, perturbing the hydrophobic lipid organization to a lesser extent than TAM [10]. Therefore, the higher inhibitor capacity of OHTAM may be a consequence of a direct interaction of its hydroxy group with polar catalytic domains of the membrane enzymes and with the lipid–protein interface. Moreover, its protonated basic tertiary amino side-chain may be prone to interact with charged head groups of phospholipids [43], affecting the lipid–protein interface structure. Processes requiring specific lipid topology are liable to perturbations and constitute putative action targets of this drug.

The potential binding sites of OHTAM on membrane proteins at the lipid–protein interface fully agreeing with its high binding to proteins [40] and high partition coefficient, may explain the higher inhibitory capacity of OHTAM ($IC_{50} = 25$ μM) towards protein kinase C as compared with TAM ($IC_{50} = 100$ μM) [8] and the stronger inhibition of Ca^{2+}-pump of SR membranes induced by OHTAM (unpublished data). Furthermore, this proposed location of the drug in lipid–protein interfaces may also account for the increased OHTAM affinity towards ER [14] and the highest partition into native membranes with the highest protein/lipid ratio [9].

In conclusion, the metabolite OHTAM as well the promoter drug TAM itself interact with membrane phospholipids perturbing the bilayer structure. However, the effects of OHTAM are stronger in the outer bilayer region whereas TAM affects preferentially the bilayer interior. These findings indicate that OHTAM is displaced mainly to the outer regions of the bilayer and TAM to the hydrophobic core of the bilayer. This specific partitioning at different depths across the bilayer thickness may be involved in the pathway by which OHTAM and TAM reach specific target receptors, transport and regulatory proteins and enzymes located at different membrane depths, contributing to the multiple cellular effects and action mechanisms of this antiestrogenic promoter drug involved in the inhibition of cell proliferation.

Acknowledgement

This work was supported by Centro de Estudos Farmacêuticos, Universidade de Coimbra.

References

1 Pavlik, E.J., Nelson, K., Srinivasan, S., Powell, D.E., Kenady, D.E., DePriest, P.D., Gallion, H.H. and Van Nagell, J.R., Jr. (1992) Cancer Res. 52, 4106–4112.
2 Jordan, V.C. (1990) Breast Cancer Res. Treat. 15, 125–136.
3 Nayfield, S.G., Karp, J.E., Ford, L.G., Dorr, F.A. and Kramer, B.S. (1991) J. Natl. Cancer Inst. 83, 1450–1459.
4 Lazier, C.B. and Bapat, B.V. (1988) J. Steroid Biochem. Mol. Biol. 31, 665–669.
5 Gilbert, J., Miquel, J.F., Précigoux, G., Hospital, M., Raynaud, J.P., Michel, F. and Paulet, A.C. (1983) J. Med. Chem. 26, 683–699.
6 Law, H.-Y. (1984) Biochem. Biophys. Res. Commun. 118, 27–32.
7 Barrera, G., Screpanti, I., Paradisi, L., Parola, M., Ferreti, C., Vacca, A., Farina, A., Dianzani, M.U., Frati, L. and Gulino, A. (1986) Biochem. Pharmacol. 35, 2984–2986.
8 O'Brian, C.A., Liskamp, R.M., Solomon, D.H. and Weinstein, I.B. (1986) J. Natl. Cancer Inst. 76, 1243–1246.
9 Custódio, J.B.A., Almeida, L.M. and Madeira, V.M.C. (1991) Biochem. Biophys. Res. Commun. 176, 1079–1085.
10 Custódio, J.B.A., Almeida, L.M. and Madeira, V.M.C. (1993) Biochim. Biophys. Acta 1150, 123–129.
11 Mani, C. and Kupfer, D. (1991) Cancer Res. 51, 6052–6058.
12 Blankson, E.A., Ellis, S.W., Lennard, M.S., Tucker, G.T. and Rogers, K. (1991) Biochem. Pharmacol. 42, S209–S212.
13 Borgna, J.-L. and Rochefort, H. (1981) J. Biol. Chem. 256, 859–868.
14 Wilson, S., Ruenitz, P.C. and Ruzicka, J.A. (1992) J. Steroid Biochem. Mol. Biol. 42, 613–616.
15 Bond, J.P., Sasson, S. and Notides, A.C. (1992) Arch. Biochem. Biophys. 296, 583–591.
16 Wiseman, H., Laughton, M.J., Arnstein, H.R.V., Cannon, M. and Halliwell, B. (1990) FEBS Lett. 236, 192–194.
17 Antunes-Madeira, M.C., Almeida, L.M. and Madeira, V.M.C. (1991) Pestic. Sci. 33, 347–357.
18 Troffer, P.J. and Storch, J. (1989) Biochim. Biophys. Acta 982, 131–139.
19 Madeira, V.M.C. and Antunes-Madeira, M.C. (1976) Ciênc. Biol. (Coimbra) 2, 265–291.
20 Gornall, A.G., Bardawill, C.J. and David, M.M. (1949) J. Biol. Chem. 47, 2387–2393.
21 Bartlett, G.R. (1958) J. Biol. Chem. 234, 466–468.
22 Böttcher, C.J.F., Van Gent, C.M. and Pries, C. (1961) Anal. Chim. Acta 24, 203–204.
23 Jurado, A.S., Almeida, L.M. and Madeira, V.M.C. (1991) Biochem. Biophys. Res. Commun. 176, 356–363.
24 Almeida, L.M., Vaz, W.L.C., Zachariasse, K.A. and Madeira, V.M.C. (1982) Biochemistry 21, 5972–5977.
25 Antunes-Madeira, M.C. and Madeira, V.M.C. (1990) Biochim. Biophys. Acta 1023, 469–474.
26 Andrich, M.P. and Vanderkooi, J.M. (1976) Biochemistry 15, 1257–1261.
27 Zachariasse, K.A.,Vaz, W.L.C., Sotomayor, C. and Kühnle, W. (1982) Biochim. Biophys. Acta 688, 323–332.
28 Almeida, L.M., Vaz, W.L.C., Zachariasse, K.A. and Madeira, V.M.C. (1984) Biochemistry 23, 4714–4720.
29 Mulders, F., Van Langen, H., Van Ginkel, G. and Levine, Y.K. (1986) Biochim. Biophys. Acta 859, 209–218.
30 Shinitzky, M. and Barenholz, Y. (1977) J. Biol. Chem. 249, 2652–2657.
31 Litman, B.J. and Barenholz, Y. (1982) Methods Enzymol. 81, 678–685.
32 Jain, M.K. and Wu, N.M. (1977) J. Membr. Biol. 34, 157–201.
33 Wright, S.E. and White, J.C. (1986) Biochem. Pharmacol. 35, 2731–2735.
34 Antunes-Madeira, M.C., Almeida, L.M. and Madeira, V.M.C. (1990) Biochim. Biophys. Acta 1022, 110–114.
35 O'Leary, T.J., Ross, P.D. and Levin, I.W. (1984) Biochemistry 23, 4636–4641.
36 Lien, E.A., Solheim, E. and Ueland, P.M. (1991) Cancer Res. 51, 4837–4844.
37 Mouritsen, O.G. and Jorgensen, K. (1992) BioEssays 14, 129–136.
38 Van Blitterswijk, W.J. (1984) In Physiology of Membrane Fluidity (Shinitzky, M., ed.), Vol. II, pp. 53–83, CRC Press, Boca Raton, FL.
39 McIntosh, T.J. (1978) Biochim. Biophys. Acta 513, 43–58.
40 Oosbree, T.R.V., Olsen, M.R., Tate, A.C., Jordan, V.C. and Mueller, G.C. (1984) Mol. Cell. Endocrinol. 35, 143–149.
41 Lopes, M.C.F., Vale, M.G. and Carvalho, A.P. (1990) Cancer Res. 50, 2753–2758.
42 Zakim, D., Kavecansky, J. and Scarlata, S. (1992) Biochemistry 31, 11589–11594.
43 Bignon, E., Pons, M., Doré, J.-C., Gilbert, J., Ojasoo, T., Miquel, J.-F., Raynaud, J.-P. and Paulet, A.C. (1991) Biochem. Pharmacol. 42, 1373–1383.