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LIPID MEMBRANE-ESTROGEN INTERACTION: MOLECULAR AND BIOPHYSICAL PERSPECTIVE: A MINI OVERVIEW

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ABSTRACT

Molecular and biophysical characterization of estrogen (ES)-lipid membrane interactions has been studied through various angles in recent years. Membrane interaction of ES, bisphenol-A (BPA), flavanones/ flavonoids, tamoxifen(TAM); ES-membrane interaction in cellular membranes of RBCs, bacteria, malignant cells; and ES-membrane interaction involved in anticancer and other drug therapies are the important issues. The ES membrane receptors (mERs) and modulators and membrane binding sites for ES are some of the highly important aspects for understanding the future foundations for molecular/ biophysical medicine. Membrane-ES interaction studies provide fascinating plans for drug therapies/ anticancer therapy, and outcomes for the treatment of medical disorders especially cancer via uncovering ES membrane localization/ binding sites and role of membrane ES in regulating metabolic physiological/ pathophysiological processes. Few of our previous studies suggested E2-membrane interaction as a possible mechanism in women with catamenial epilepsy. The present article is mainly related to involvement of ES while interacting with lipid part of the membrane. Further studies will clarify the other unknown aspects of ES-membrane interaction and its various potential pharmaceutical, medical, biological, chemical and physicochemical/ chemicophysical applications.

KEYWORDS: Estrogen-membrane interaction, Tamoxifen; Bisphenol A, RBCs, Bacteria, Cancer, Anticancer therapy, ES membrane receptors/modulators/binding sites.

List of abbreviations

Alpha-T: alpha-tocopherol
ATR-FTIR: Attenuated total reflection Fourier transform infrared
BPA: Bisphenol A
BSA: Bovine serum albumin
CDKIs: Cyclin-dependent kinase inhibitors
CE: Coefficient of eximerization
CF: Carboxyfluorescein
DMAC: Dimethylaminochalcone
DPH: 1,6-diphenyl-1,3,5-hexatriene
DPH-PA: DPH-propionic acid derivative
DPPC: Dipalmitoylphosphatidylcholine (1,2-dipalmitoyl-sn-glycero-3-phosphocholine)
DPPC MLV: Dipalmitoylphosphatidylcholine multilammelar vesicles
DPPtdCho: Dipalmitoylphosphatidylcholine
DSC: Differential scanning calorimetry
E2: Estradiol (17beta-Estradiol)
EPC: Egg yolk phosphatidylcholine
EPR: Electron paramagnetic resonance
ER: Estrogen receptor
ES: Estradiol
ESR: Electron spin resonance (EPR)
FTIR spectroscopy: Fourier-transform infrared spectroscopy
GPER1: G-protein-coupled receptor 1
HCVECs: Human cerebral vascular endothelial cells
LUV: Large unilamellar vesicles
(1)H NMR: Proton nuclear magnetic resonance
MD: Molecular dynamics
MDS: Molecular dynamics simulation
2-Me: 2-methoxyestradiol
mERs: Membrane estrogen-receptors
MLV: Multilammelar vesicles
4-OHTAM: 4-hydroxytamoxifen
PDT: Photodynamic therapy
RBCs: Red blood cells
s-BLM: Supported bilayer lipid membrane
SERMs: Selective estrogen receptor modulators
SHG: Second harmonic generation
TAM: Tamoxifen
Tm: Phase transition temperature
TMA-DPH: 1-[4-[trimethylammonium)phenyl]-6-phenylhexa-1,3,5-triene

INTRODUCTION

Estrogen (ES) is a female hormone (E2 or 17beta-Estradiol- a potent form of estrogen present in high levels during normal reproductive age in females) and it is present in males but in lower levels. However, it performs important functions through estrogen receptors (ERs) present as nuclear estrogen-receptors (ERα and ERβ) and membrane estrogen-receptors (mERs). ES plays significant role in menstrual cycle and other reproductive functions and a variety of other functions both in males and females e.g., cognitive functions wherein membrane interactions, intercellular signaling, and neurosteroid actions (Hussain and Zahir, 2012) influence excitability, learning and memory, but effective therapeutic interventions for hormone/ drug developments using E2 have yet to be realized for enhancing the cognitive functions (Luine, 2014).

Summarized view of information for molecular and biophysical aspects of E2-membrane are given in Table 1. Molecular and biophysical characterization of ES-lipid membrane interactions have been studied through various angles in recent years (Pawlęga *et al*., 2014; Pawlikowska-Pawlęga *et al*., 2014; Stokes and Conboy, 2014; Tsyrlina *et al*., 2014; Vogel *et al*., 2014; Wesołowska *et al*., 2014; Saczko *et al*., 2015; Broniatowski *et al*., 2016; Chen *et al*., 2016). It was found that ES and especially E2 membrane interaction studies (Dimitrov and Lalchev, 1998; Golden *et al*., 1998; Inouye *et al*., 2000; El Maghraby *et al*., 2005; Scheidt *et al*., 2010) and the reports related to BPA (Bisphenol A) entrance in membrane, pore formation, and membrane damage/ structural changes (Vogel, 2013; Broniatowski *et al*., 2016; Chen *et al*., 2016) have provided enormous potential information. Some of the pertinent studies show the importance of bioflavonoid membrane insertion, and especially the significance of genistein in the treatment of cancer and other diseases (Kużdżał *et al*., 2011; Pawlikowska-Pawlega *et al*., 2012; Raghunathan *et al*., 2012; Pawlikowska-Pawlęga *et al*., 2014; Wesołowska *et al*., 2014).

Studies were carried out on tamoxifen (TAM) for DPPC MLV/ EPC LUV -TAM interaction, TAM and change in hemolysis of human RBCs/ hemolytic anemia, influence of low concentrations of alpha-T(alpha-tocopherol), bacterial toxicity, membrane perturbation, influence of Ca^{2+} and Mg^{2+} on interaction/cytotoxicity of TAM and role of chlorpromazine interaction for cytotoxic effect of TAM (Luxo *et al*., 1996, 2001; Luxo *et al*., 1999; Cruz Silva *et al*., 2000; Engelk *et al*., 2001; Monteiro *et al*., 2003; Yde *et al*., 2009).

Erythrocyte membrane-ES interaction studies (Cruz Silva *et al*., 2000; Kużdżał *et al*., 2011; Pawlikowska-Pawlęga *et al*., 2014; Tsyrlina *et al*., 2014) were found related to: TAM, alpha-T and RBC hemolysis, anticancer action, genistein interaction and intracellular changes, genistein modifying the domain structure of membranes in RBCs, less E2 dependence, higher viscosity and less CE (coefficient of eximerization) of pyrene in protein-lipid and lipid-lipid layer of erythrocyte membranes in breast cancer patients, and increase in microviscosity for identifying tumor of ES dependent type.

Bacterial membrane ES interaction provided information about: bacterial membrane model and molecular mechanisms of TAM-membrane physical interactions high drug partitioning in bacterial lipid membranes and TAM induced growth perturbation, influence of the toxic effects, TAM toxicity on growth/ respiratory activity in B. stearothermophilus, role of Ca^{2+} or Mg^{2+} in causing TAM induced cytotoxicity, and TAM induced physical ordering instead of disordering by Ca^{2+} (Luxo *et al*., 1996, 2001; Luxo *et al*., 1999; Monteiro *et al*., 2003).

The ES receptors/ modulator studies (Acconcia *et al*., 2005; Xia *et al*., 2010; Tu and Jufri, 2013; Stokes and Conboy, 2014) provided insight of: HCVECs (human cerebral vascular endothelial cells) model and pathogenesis of cerebral aneurysm formation in menopausal/ postmenopausal woman, E2 regulation of palmitoylation of ERalpha interaction with membrane caveolin-1/signaling/ cell proliferation pathways & palmitoylation, and membrane adsorption with TAM and its metabolites. Membrane ES in malignancy/ anticancer therapy, role of ES on viscosity, CE of pyrene in protein-lipid and lipid-lipid layer of membranes in breast cancer patients, microviscosity for identifying tumor of hormone dependent type, and oxidative modification via oxidative stress in malignant cells were studied (Monteiro *et al*., 2003; Tsyrlina *et al*., 2014; Saczko *et al*., 2015). Membrane ES interaction in drug therapies (Monteiro *et al*., 2003; Oren *et al*., 2004; El Maghraby *et al*., 2005; Vogel *et al*., 2014; Saczko *et al*., 2015) were studied by determining diffusion of E2 across biomembranes, oxidative modification, molecular localization, and design for specific E2-HDL-targeted drug therapies. Membrane binding sites for estrogen were studied in plasmatic hepatocyte membranes and radioiodinated E2 BSA conjugate-neuronal membrane binding (Sergeev and Denisov, 1978; Suleĭmanov *et al*., 1985; Zheng *et al*., 1996).

The observations that estrone lessens the constant of probe binding with membrane DMAC proportional to the distribution coefficient of estrone in lipids-water system, uncovery that recognition system as binding of E2 is either with membrane glycoproteins or diffusion of ES into lipid membrane phase, and E2-BSA conjugates linkage at C-6 position demonstrating the existence of specific membrane binding sites for E2 in several regions of brain are newer facets in understanding the precise ES-membrane interaction (Sergeev and Denisov, 1978; Suleĭmanov *et al*., 1985; Zheng *et al*., 1996).

Few of our previous studies suggested E2-membrane interaction as a possible mechanism in women with catamenial epilepsy (Hussain *et al*., 1988, 1989; Hussain, 1991; Hussain *et al*., 2006; Hussain, 2010). The present article is mainly related to involvement of ES while interacting with lipid part of the membrane. Further studies will clarify the other unknown aspects of ES-membrane interaction and its various potential pharmaceutical, medical, biological, chemical and physicochemical/ chemicophysical applications.

ESTROGEN AND RELATED COMPOUNDS

The E2-membrane interaction studies were carried out by several investigators (Dimitrov and Lalchev, 1998; Golden *et al*., 1998; Inouye *et al*., 2000; El Maghraby *et al*., 2005; Scheidt *et al*., 2010). Summarizes the results of these studies is given in Table 1. The interaction of ES and cholesterol with monolayer DPPC (dipalmitoylphosphatidylcholine) showed that E2- DPPC monolayer interaction forces were found dependent on DPPC phase state (Dimitrov and Lalchev, 1998). The lecithin bilayer-E2 interaction examined in physiological like conditions by small-angle X-ray diffraction characterized E2 partition to specific sites in the membrane bilayer (Golden *et al*., 1998).

Table 1. Molecular and biophysical characterization of the lipid membrane-estrogen interaction.

Estrogens	Characterization/ Molecular and Biophysical Mechanisms	References
ES and related compounds	- E2 upper chain broad membrane distribution - E2-DPPC monolayer interaction on DPPC phase state dependence, site partition, Ras/Raf activation and acyl chain interaction - BPA and membrane fluidity/damage/ cytotoxicity - Bioflavonoids go close to carbonyls, bilayer thicknesses/ softening bilayers - Genistein modifies the intracellular/membrane domain structure and serves treatment for cancer and other disorders - DPPC bilayers intercalated with prenylated chalcones and flavanones correlating with lipophilicity and molecular shape of compounds, and decrease in melting temperature - DPPC MLV/ EPC LUV -TAM interaction and broadening of the phase transition profile/ TAM decrease in DPPC MLV, increase in lipid bilayer order in the outer bilayer region in EPC LUV and TAM-induced CF release - TAM and change in hemolysis/cytotoxicity, anticancer action - High drug partitioning in the bacterial lipid membranes causes perturbation - Ca(2+) or Mg(2+) affects the interaction/cytotoxicity of TAM. TAM- a lipophilic anticancer drug - Chlorpromazine interaction increases cytotoxic effect of TAM through ER-mediated mechanism	(Luxo *et al*., 1996, 2001; Dimitrov and Lalchev, 1998; Golden *et al*., 1998; Luxo *et al*., 1999; Cruz Silva *et al*., 2000;Inouye *et al*., 2000; Engelk *et al*., 2001; Monteiro *et al*., 2003; El Maghraby *et al*., 2005; Yde *et al*., 2009; Scheidt *et al*., 2010; Kużdżał *et al*., 2011; Pawlikowska-Pawlega *et al*., 2012; Raghunathan *et al*., 2012; Vogel, 2013; Pawlikowska-Pawlęga *et al*., 2014; Wesołowska *et al*., 2014; Broniatowski *et al*., 2016; Chen *et al*., 2016)
Cellular membrane ES and ER modulation	- TAM, alpha-T and RBC hemolysis, anticancer action - In less steroid hormone dependence, higher viscosity and less CE of pyrene in protein-lipid and lipid-lipid layer of erythrocyte membranes in breast cancer patients - Increase in microviscosity for identifying tumor of E2 dependent type - High drug partitioning in bacterial lipid membranes, and TAM induced growth perturbation - TAM induced physical ordering instead of disordering by Ca(2+) - TAM- a lipophilic anticancer drug; In less steroid hormone dependence, higher viscosity and less CE of pyrene in protein-lipid and lipid-lipid layer of membranes in breast cancer patients - Drug-membrane interaction and lipophilic E2 - acyl chains - Free diffusion of E2 across biomembranes was determined by calculating free energy of interacting E2 interaction - E2 may rapidly cross transbilayer barriers; A design for specific E2-HDL-targeted drug therapies - HCVECs model and pathogenesis of cerebral aneurysm formation - 17β-estradiol-GPER1 interaction was dose responsive; xenoestrogens (bisphenol A and 4-nonylphenol etc.) - E2 regulates palmitoylation of ERalpha interaction with membrane caveolin-1/signaling/ cell proliferation pathways & palmitoylation - Membrane adsorption with TAM and its metabolites important for understanding SERM action in vivo - E2 has been suggested to be related partly in our studies interacting with membrane for its mode of action in menstrual cycle related epilepsy, ischemic disorders and kindling model, reproductive disorders and experimental metabolic studies	(Hussain *et al*., 1988, 1989; Hussain, 1991; Hussain, 1993 a, b; Abbas *et al*., 1995; Inam *et al*., 1995; Khan *et al*., 1995; Masood *et al*., 1995; Luxo *et al*., 1996, 2001; Luxo *et al*., 1999; Cruz Silva *et al*., 2000; Monteiro *et al*., 2003; Oren *et al*., 2004; Acconcia *et al*., 2005; El Maghraby *et al*., 2005; Hussain *et al*., 2006; Hussain, 2010; Xia *et al*., 2010; Kużdżał *et al*., 2011; Hussain and Zahir, 2012; Rehman *et al*, 2012; Rehman *et al*., 2013; Tu and Jufri, 2013; Pawlikowska-Pawlęga *et al*., 2014; Rehman *et al*., 2014; Stokes and Conboy, 2014; Tsyrlina *et al*., 2014; Vogel *et al*., 2014; Saczko *et al*., 2015)
ES binding sites	- Estrone lessens the constant of probe binding with membrane DMAC proportional to the distribution coefficient of estrone in lipids-water system, but estrone did not produce this effect in plasmatic hepatocyte membranes - Interaction/ accumulation of 3H-E2 into plasmatic membranes of uterine cells showed recognition system as binding of E2 either with membrane glycoproteins or diffusion of estrogen into lipid membrane phase - A dioiodinated E2 BSA conjugate-neuronal membrane binding showed that E2-BSA conjugates linked at C-6 position demonstrate the existence of specific membrane binding sites for E2 in several regions of the rat brain	(Sergeev and Denisov, 1978; Suleĭmanov *et al*., 1985; Zheng *et al*., 1996).

Abbreviations are given in the text as “List of abbreviations”

Solid-state NMR methods (2)H NMR, and (1)H magic-angle spinning NMR) for E2-membrane interaction indicate that E2 is broadly distributed maximally in upper chain region of the membrane, and being highly dynamic in lipid membranes may rotates since it manifests two polar hydroxyl groups on either side of the molecule (Scheidt *et al*., 2010). Furthermore, Ras-liposome localization revealed that membrane localization is essentially required to Ras for forming a dimer, which is essential, although not sufficient, for the activation of Raf-1 (Inouye *et al*., 2000). Further work indicated that drug-membrane interaction study correlating with PSA (polar surface area) determined lipophilic ES- acyl chains of the lipid membrane interaction (El Maghraby *et al*., 2005).

The BPA (an ES mimicking xenoestrogen that behaves like hormone possessing properties) interacts with lipid membranes (Broniatowski *et al*., 2016; Chen *et al*., 2016). Hence, BPA should be checked in food containers and consumable items (Vogel, 2013) since it may cause cytotoxicity, birth defects, cancerous tumors, neoteny and several human toxicities (Chen *et al*., 2016). The molecular dynamics simulation study shows that BPA enters membrane, aggregates, and forms membrane pores causing cell membrane damage leading possibly to cytotoxicity (Chen *et al*., 2016). Using the techniques of anionic phospholipid Langmuir monolayer model membranes, surface potential measurements, Grazing incidence X-ray diffraction and Brewster angle microscopy, it was found that BPA having estrogenic activities interact with biomembranes and causes risk for public / environmental health via changing the structure and fluidity of membranes (Broniatowski *et al*., 2016).

Bioflavonoids (genistein and daidzein)-lipid membranes (DOPC and diphytanoy lPC) interaction study provides insight that bioflavonoids insert into the hydrocarbon region in the lipid membranes close to carbonyls of lipids, decrease bilayer thicknesses and soften bilayers (Raghunathan *et al*., 2012). Genistein-erythrocyte/ model membranes (dimyristoyl- and dipalmitoylphosphatidylcholine) interaction studied by ESR (electron spin resonance) / fluorescence spectroscopy revealed that genistein modifies the domain structure of membranes by intercalating mainly into lipid headgroup-region and to some extent into the interface of polar-apolar region, but to only quite a little extent into the hydrophobic core (Kużdżał *et al*., 2011).

Genistein-membrane (DPPC liposomes) interactions alter the membrane properties leading to intracellular changes, and hence, genistein can be employed pharmacologically for the treatment of cancer or other diseases (Pawlikowska-Pawlega *et al*., 2012). Genistein-membrane interaction carried out by EPR (Electron paramagnetic resonance) and (1)H NMR (proton nuclear magnetic resonance) for egg yolk lecithin liposomes and human erythrocyte membrane interaction with genistein, light / transmission electron / scanning electron microscopy and fluorescent techniques revealed that influence of genistein-lipid membrane interaction on membrane organization and biophysical properties correlated with intracellular changes (Pawlikowska-Pawlęga *et al*., 2014). Another study reveals that interaction of DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine) membranes with prenylated chalcones and flavanones was studied employing fluorescence and ATR-FTIR (attenuated total reflection-Fourier transform infrared) spectroscopies and DSC (differential scanning calorimetry) and that showed DPPC bilayers intercalating with prenylated chalcones and flavanones in correlation with lipophilicity and molecular shape of compounds and decrease in melting temperature (Wesołowska *et al*., 2014).

DPPC MLV/ EPC LUV -TAM interaction study showed broadening of the phase transition profile, decrease in Tm in DPPC MLV and increase in lipid bilayer order in the outer bilayer region in EPC LUV indicating the TAM-induced CF release due to permanent perturbation/ disruption/ transient hole formation in the lipid bilayer (Engelk *et al*., 2001).

Studies were carried out on TAM for DPPC MLV/ EPC LUV -TAM interaction, TAM and change in hemolysis of human RBCs/ hemolytic anemia, influence of low concentrations of alpha-T (alpha-tocopherol), bacterial toxicity, membrane perturbation, influence of Ca(2+) and Mg(2+) on interaction/ cytotoxicity of TAM and role of chlorpromazine interaction for cytotoxic effect of TAM (Luxo *et al*., 1996, 2001; Luxo *et al*., 1999; Cruz Silva *et al*., 2000; Engelk *et al*., 2001; Monteiro *et al*., 2003; Yde *et al*., 2009).

Influence of the toxic effects of TAM on bacterial growth/ respiratory activity occurs via TAM-membrane interaction in B. stearothermophilus (Luxo *et al*., 2001). High partitioning of TAM was observed in the *Bacillus* stearothermophilus lipid membranes, and TAM was found to impair the bacterial growth perturbation in bacterial membrane lipids (Luxo *et al*., 1996). A bacterial model system of a strain of *Bacillus* stearothermophilus revealed that Ca(2+) or Mg(2+) affects the cytotoxicity of TAM and interaction of membrane with TAM. Physical ordering of bacterial lipid liposomes instead of disordering caused by TAM occurs in response to the addition of Ca(2+) that indicates TAM-induced growth impairment occurred due to membrane perturbations (Luxo *et al*., 1999).

TAM/ 4-OHTAM (4-hydroxytamoxifen)-bacterial membrane interactions were characterized in a strain of thermophilic eubacterium- *Bacillus* stearothermophilus as quite informative, economical and simple for understanding the molecular mechanisms of membrane physical interactions of TAM-a lipophilic anticancer drug. (Monteiro *et al*., 2003). Interaction of chlorpromazine in model membranes of unilamellar liposomes reveals chlorpromazine- lipid bilayers interaction quite strong and increased permeability indicating that chlorpromazine seems promising chemosensitizing compound for increasing the extent of cytotoxic effect of TAM through ER-mediated mechanism (Yde *et al*., 2009).

CELLULAR MEMBRANE 'ES' AND 'ER' MODULATION

Topic of cellular membrane ES interactions and ER modulation has been summarized in Table 1. It was noted that anticancer action might be due to TAM causing cellular changes, membrane and structural derangement/ cytotoxicity (Cruz Silva *et al*., 2000). Membrane microviscosity and CE measurement carried out in breast cancer patients showed higher viscosity and less CE of pyrene in protein-lipid and lipid-lipid layer of erythrocyte membranes in less steroid hormone dependence, and it was considered that increase in microviscosity might be an additional factor for identifying tumor of ES dependent type (Tsyrlina *et al*., 2014). High partitioning of TAM leading to bacteria membrane growth/respiratory disturbances and modulation of Ca^{2+} addition clarified that TAM-impaired action was in response to membrane perturbances (Luxo *et al*., 1996, 1999, 2001).

The HCVECs (human cerebral vascular endothelial cells) seem nice cellular model employing immunocytochemistry for understanding the pathogenesis of cerebral aneurysm formation in menopausal/ postmenopausal woman, where interaction between E2 and GPER1 (located in plasma membrane) was found dose responsive (Tu and Jufri, 2013). E2 has been suggested to be related partly in our studies interacting with membrane for its mode of action in menstrual cycle related epilepsy (Hussain *et al*., 1988, 1989; Hussain, 1991; Hussain *et al*., 2006; Hussain, 2010), ischemic disorders and kindling model (Hussain and Zahir, 2012), stroke (Hussain, 1993a, b) reproductive disorders (Rehman *et al*, 2012; Rehman *et al*., 2013 ; Rehman *et al*., 2014) and experimental metabolic studies (Abbas *et al*., 1995; Inam *et al*., 1995; Khan *et al*., 1995; Masood *et al*., 1995).

Nanostructure electrochemical biosensor developed for studying the ES-ER binding in Au modified s-BLM (supported bilayer lipid membrane) was found to be able to detect accurately the natural estrogen 17beta-estradiol and other xenoestrogens (bisphenol A and 4-nonylphenol etc.) with the best sensitivity (Xia *et al*., 2010). E2 regulates palmitoylation of ERalpha interaction with caveolin-1 protein in plasma membrane, signaling and cell proliferation pathways and reducing palmitoylation of ERalpha and caveolin-1 interaction in relation to time and dose dependence (Acconcia *et al*., 2005). Another report clarifies that SERMs-lipid membrane interaction using label-free method of SHG for lipid phase, packing density, membrane partitioning, and cholesterol content indicated that membrane adsorption with TAM and its metabolites may be important for understanding SERM *In vivo* action (Stokes and Conboy, 2014).

Membrane microviscosity and CE (coefficient of eximerization) measurement carried out in breast cancer patients showed higher viscosity and less CE of pyrene in protein-lipid and lipid-lipid layer of erythrocyte membranes in less steroid hormone dependence, and it was considered that increase in microviscosity might be an additional factor for identifying tumor of hormone dependent type (Tsyrlina *et al*., 2014).

The 2-Me (2-methoxyestradiol)-lipid membrane interaction was caused via Photodynamic therapy (PDT) in ovarian carcinoma and human breast adenocarcinoma cell lines and oxidative modification via oxidative stress in malignant cells was employed as anticancer therapy (Saczko *et al*., 2015). Drug-membrane interaction study correlating with PSA (polar surface area) determined lipophilic ES- acyl chains of the lipid membrane interaction (El Maghraby *et al*., 2005). Free diffusion of E2 across biomembranes was determined by calculating the free energy of E2 interaction with lipid membranes, and it was found interesting for therapeutic applications that that E2 may rapidly cross transbilayer barriers (Oren *et al*., 2004).

The NMR, MD simulations, and analytic theory for studying the E2/E2 oleate–membranes/HDLs orientation showed that oleoyl chain firmly inserted into the membrane/HDLs suggests only lipid-E2 interactions determining the localization of the molecule and providing a design for specific E2-HDL-targeted drug therapies (Vogel *et al*., 2014).

ESTROGEN BINDING SITES

The Table 1 identifies briefly the studies carried out related to ES membrane binding sites. Estrone lessens the constant of probe binding with DMAC in lipid membranes proportional to the distribution coefficient of estrone in lipids-water system, but estrone did not produce this effect in plasmatic membranes of the hepatocyte (Sergeev and Denisov, 1978).

Interaction/ accumulation of 3H-E2 into plasmatic membranes of the rat uterine cells showed recognition system as binding of E2 either with membrane glycoproteins or diffusion of ES into lipid membrane phase (Suleĭmanov *et al*., 1985). Radioiodinated E2 BSA conjugate-neuronal membrane binding showed that E2-BSA conjugates linked at C-6 position demonstrated the existence of specific membrane binding sites for E2 in several regions of the rat brain (Zheng *et al*., 1996).

CONCLUSIONS

The ES-lipid membrane interaction studies clarify the upper chain broad membrane distribution, E2-DPPC monolayer interaction on DPPC phase state dependence and E2 partition for specific sites, Raf-1 activation, and significance of lipophilic estradiol (ES)-acyl chains in drug-membrane interaction (Dimitrov and Lalchev, 1998; Golden *et al*., 1998; Inouye *et al*., 2000; El Maghraby *et al*., 2005; Scheidt *et al*., 2010). Furthermore, it is quite important

information that the significance of BPA interaction relates to cytotoxicity, membrane structure and fluidity (Vogel, 2013; Broniatowski *et al*., 2016; Chen *et al*., 2016).

Another interesting series of investigations were the bioflavonoids insertion into the hydrocarbon region close to carbonyls of lipids, decrease the bilayer thicknesses and softening of bilayers, genistein modifying the domain structure of membranes by intercalating mainly into lipid headgroup and to some extent into the interface of polar-apolar region, but to only quite a little extent into the hydrophobic core, and its interactions altering the membrane properties leading to intracellular changes (Kużdżał *et al*., 2011; Pawlikowska-Pawlega *et al*., 2012; Raghunathan *et al*., 2012; Pawlikowska-Pawlęga *et al*., 2014; Wesołowska *et al*., 2014).

DPPC MLV/ EPC LUV -TAM interaction and broadening of the phase transition profile/ TM decrease in DPPC MLV, increase in lipid bilayer order in the outer bilayer region in EPC LUV indicating TAM-induced CF release, effect of Ca^{2+} or Mg^{2+} on the interaction/cytotoxicity of TAM, physical ordering of bacterial lipid liposomes instead of disordering caused by TAM with addition of Ca^{2+}; TAM- a lipophilic anticancer drug and finally the chlorpromazine interaction increasing the extent of cytotoxic effect of TAM through ER-mediated mechanism (Luxo *et al*., 1996, 2001; Luxo *et al*., 1999; Cruz Silva *et al*., 2000; Engelk *et al*., 2001; Monteiro *et al*., 2003; Yde *et al*., 2009) are quite fascinating investigations.

Moreover, it is quite valuable information that E2-GPER1 interaction is dose responsive, and E2 regulates palmitoylation of ERalpha interaction with membrane caveolin-1/signaling/ cell proliferation pathways & palmitoylation (Acconcia *et al*., 2005; Xia *et al*., 2010; Tu and Jufri, 2013; Stokes and Conboy, 2014).

Another insight that TAM is a lipophilic anticancer drug having higher viscosity and less CE of pyrene in protein-lipid and lipid-lipid layer of membranes in breast cancer patients in less estrogen dependence and increase in microviscosity and oxidative modification via oxidative stress in malignant cells seems promising as a treatment for cancer (Monteiro *et al*., 2003; Tsyrlina *et al*., 2014; Saczko *et al*., 2015).

Investigation of Drug-membrane interaction and lipophilic ES- acyl chains, free diffusion and rapid crossing of E2 across biomembranes and firm insertion of oleoyl chain into the membrane/HDLs suggests that only lipid-E2 interactions determine the localization of the molecule and provides a design for specific E2-HDL-targeted drug therapies (Monteiro *et al*., 2003; Oren *et al*., 2004; El Maghraby *et al*., 2005; Vogel *et al*., 2014; Saczko *et al*., 2015).

Membrane interaction of ES, bisphenol, flavonones/ flavinoids, TM; ES-membrane interaction in cellular membranes of RBCs, bacteria, malignant cells; and ES-membrane interaction involved in anticancer and other drug therapies are the important issues. The ES membrane receptors and modulators and membrane binding sites for ES are some of the highly important aspects for understanding the future foundations for molecular/ biophysical medicine. Membrane- ES interaction studies provide fascinating plans for drug therapies/ anticancer therapy, and outcomes for the treatment of medical disorders especially cancer via uncovering ES membrane localization/ binding sites and role of membrane ES in regulating metabolic physiological/ pathophysiological processes. It is verified by few of our previous studies suggesting E2-membrane interaction a possible mechanism of seizure occurrence patterns in women with precatamenial epilepsy.

Our studies suggesting the E2-membrane interactions in various medical disorders and metabolic processes (Hussain, 1991, 2010; Rehman *et al*., 2014) further point out toward additional studies be conducted for understanding other important facets related to ES-membrane interaction and promising applications.

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