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Spectroscopic and calorimetric studies on trazodone hydrochloride–phosphatidylcholine liposome interactions in the presence and absence of cholesterol

Dilek Yonar, M. Maral Sünnetçioğlu *

Hacettepe University, Dept. of Physics Engineering, 06800 Beytepe, Ankara, Turkey

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ABSTRACT

The interaction of antidepressant drug trazodone hydrochloride (TRZ) with dipalmitoyl phosphatidylcholine (DPPC) multilamellar liposomes (MLVs) in the presence and absence of cholesterol (CHO) was investigated as a function of temperature by using Electron Paramagnetic Resonance (EPR) spin labeling, Fourier Transform Infrared (FTIR) Spectroscopy and Differential Scanning Calorimetry (DSC) techniques. These interactions were also examined for dimyristoyl phosphatidylcholine (DMPC) multilamellar liposomes by using Electron Paramagnetic Resonance (EPR) spin labeling technique. In the EPR spin labeling studies, 5- and 16-doxyl stearic acid (5-DS and 16-DS) spin labels were used to monitor the head group and alkyl chain region of phospholipids respectively. The results indicated that TRZ incorporation causes changes in the physical properties of PC liposomes by decreasing the main phase transition temperature, abolishing the pre-transition, broadening the phase transition profile, and disordering the system around the head group region. The interaction of TRZ with unilamellar (LUV) DPPC liposomes was also examined. The most pronounced effect of TRZ on DPPC LUVs was observed as the further decrease of main phase transition temperature in comparison with DPPC MLVs. The mentioned changes in lipid structure and dynamics caused by TRZ may modulate the biophysical activity of membrane associated receptors and in turn the pharmacological action of TRZ.

1. Introduction

Trazodone, known as 2-(3-(4-(3-chlorophenyl) piperazin-1-yl) propyl)-1,2,4-triazolo[4,3-a] pyridine-3(2H)-one hydrochloride (Fig. 1) is a triazolopyridine derivative. It is the first triazolopyridine derivative to be used clinically and it belongs to the group of second generation sedative, anxiolytic non-tricyclic antidepressants. It has been shown that trazodone (TRZ) is effective in patients with major depressive disorders and is generally more useful in depressive disorders associated with insomnia and anxiety. It is thought that the antidepressant properties of TRZ may be related to its action on poorly blocking serotonin reuptake and selectively blocking presynaptic receptors as well as having activity at 5-HT_1, 5-HT_2 serotonergic receptors. In addition, TRZ blocks alpha-2 adrenoceptors [1].

Hydrophobic or amphiphilic structure of TRZ as many other pharmacologically active compounds (antibiotics, antifungal, antidepressants, antihistamines, local anesthetics, anticancer drugs, etc.) causes it to feature surface active properties. Due to these properties, they tend to adsorb, aggregate and bind in different regions within a cell [2]. Transport of drugs or drug delivery systems through the cell membrane is inevitable for them to reach their targets. Therefore investigation of membrane–drug interactions is important for understanding the mechanisms of drug action, as well as developing effective drug delivery systems [3]. Due to the complexity of biological membrane structure, model membranes composed of cellular membrane lipids are used to evaluate membrane–drug interactions. Antidepressants belong to the group of amphiphilic drugs with high membrane permeability and the investigation of their interactions with biomembranes provides information about cellular processes. Recent studies have shown that antidepressant drugs cause modifications in cellular signaling [4]. Moreover, it has been known that membrane lipids participate in cellular signaling. If any alterations in the physical properties such as diffusion and fluidity occur as a result of drug–lipid interaction, this will be effective on the activity of membrane associated receptors and transport proteins. So that, drug–phospholipid interaction will be effective on the pharmaceutical efficacy of the drug.

In this study, FTIR, EPR and DSC were used to investigate the interaction of TRZ with PC liposomes. FTIR is a useful spectroscopy to characterize liposomes on a sub molecular level [5,6]. The investigation of the vibrations of individual groups provides structural information on the localized regions such as head groups and acyl chains of the bilayer. FTIR provides information regarding the effect of drug on lipid order, lipid dynamics, phase transition behavior and hydration of head group

* Corresponding author. Tel.: +90 312 2977224; fax: +90 312 2992037.
E-mail address: maral@hacettepe.edu.tr (M.M. Sünnetçioğlu).

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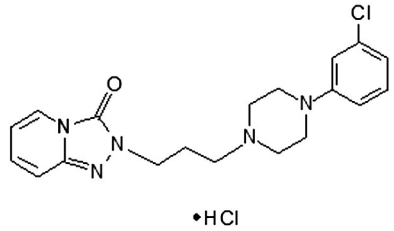

Fig. 1. Chemical structure of trazodone hydrochloride (TRZ).

and interfacial region [7–10]. DSC technique has been extensively used to study the thermotropic phase behavior of lipids in model and biological membranes [11] and to investigate the thermal changes caused by drug incorporation into phospholipid bilayers [12–14]. EPR spin labeling spectroscopy has been proven to be one of the useful techniques in the area of lipid research [15]. Spin labels are stable paramagnetic species. They are highly sensitive to the environmental changes and they provide site specific information on the dynamics, ordering and spectral components (domains) of the membranes [12,13,16–18]. Further information about the physical parameters of the domains can be obtained from simulations of EPR spectra [19,20].

Literature on trazodone hydrochloride (TRZ) showed that the studies related with drug–membrane interactions were limited. TRZ's interaction with serum albumin derived from bovine blood was investigated by using fluorescence spectroscopy and spectrophotometry methods. It was concluded that hydrophobic interactions occurred between bovine serum albumin and TRZ and also binding of TRZ to bovine serum albumin caused conformational changes in albumin [21]. Binding of TRZ to serum albumin derived from human blood was investigated using fluorescence spectroscopy by the same group. Both hydrogen bonding and hydrophobic interactions were determined to be effective in the binding of TRZ to serum albumin [22]. In a previous study, TRZ was described as multifunctional drug and the dose dependent changes in the mechanism of action were investigated [1]. The effect of various enhancers in the matrix-based transdermal formulation on the in vitro transport of TRZ across mouse and human cadaver epidermis was investigated depending on the film structure and drug concentration by scanning electron microscopy and FTIR. The passive diffusion of TRZ through human cadaver epidermis was found to be small due to its ionic behavior at the site of permeation [23].

Although there are some studies on biological membranes, there is no study of TRZ using model membranes as long as we know. Due to the importance of both membrane composition and temperature on the membrane drug interactions, in the present work, we performed EPR, FTIR and DSC studies of PC group lipids in the presence and absence of cholesterol (CHO), in the temperature range of 10–55 °C. Cholesterol is a significant constituent of many mammalian plasma membranes, where it is present at 30–50 mol% [24]. Therefore in this study 30 mol% CHO was used to mimic plasma membrane. Studies were performed mainly with MLV PC liposomes, but the effect of lamellarity was also investigated by EPR using LUV DPPC liposomes.

2. Materials and methods

2.1. Materials

1,2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) phospholipids, cholesterol (CHO), spin labels 5- and 16-doxyl stearic acid (5-DS, 16-DS) and antidepressant drug trazodone hydrochloride (TRZ) were purchased from Sigma (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). Phosphate buffered saline (PBS) (without Ca, Mg) was obtained from Dr. Zeydanlı (Dr. Zeydanlı Life Sciences, Ltd. Şti., Ankara, Turkey).

2.2. Methods

2.2.1. Electron paramagnetic resonance (EPR) spectroscopy

2.2.1.1. Sample preparation. DMPC and DPPC liposomes were prepared in the absence and presence of 30 mol% CHO. Studies were performed using 5-DS and 16-DS spin labels at 1 mol% final concentration. Only DMPC was studied using 5-DS spin label. 1, 5, and 10 mol% of TRZ were incorporated into the membranes. Phospholipids, CHO and TRZ were dissolved in chloroform stock solution containing spin label. Chloroform was evaporated first with nitrogen gas stream and samples were kept under vacuum for overnight to remove residual chloroform. The obtained dry films of the samples were hydrated with 0.15 ml of PBS at pH 7.4, vortexed at a temperature above phase transition temperature of the lipids to get multilamellar liposomes (MLVs) and then centrifuged (Eppendorf 5804-R; Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany) at 20 °C [25]. The total amount of lipids was changed between 70 and 100 mmol/L. Pellets were used in the studies. For large unilamellar liposomes (LUVs) preparation, the MLV suspensions were transferred into Avanti Mini Extruder (Avanti Polar Lipids Inc., Alabama, USA) and they were formed by passing the suspensions through polycarbonate filters (200 nm pore size). The procedure was repeated 10 times at a temperature above the phase transition temperature of the phospholipids.

Samples were mainly prepared at pH 7.4. DMPC, DPPC and their 10 mol% of TRZ incorporated samples were also prepared at pH 9.5 in order to evaluate the possible influence of pH on the distribution of fatty acid spin labels in the liposome and the effects of pH on drug–liposome interactions.

EPR measurements were performed on a Bruker EMX-131 spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) with ER4103TM cylindrical cavity using the following spectral conditions: modulation frequency 100 kHz, modulation amplitude 0.2 mT and microwave power 10 mW. Sample temperature was controlled to ± 1 °C by a Bruker VT4111 temperature controller. EPR measurements were carried out in a temperature range of 10–55 °C and repeated at least two times for each sample group. The selected temperature range covers the main phase transition temperatures (23 °C for DMPC and 41 °C for DPPC) of both lipids.

2.2.1.2. Computer simulation of EPR spectra. In the EPR studies, inner and outer hyperfine splitting values ($2A_{max}$, $2A_{min}$) can be measured from the direct evaluation of the spectra and by the use of these parameters it is possible to obtain the averaged order parameter [26]. However in the temperature dependent studies it is not always possible to determine the $2A_{min}$ values from the spectra correctly [27]. This difficulty can be overcome with the single domain spectral simulation. In the present study, single domain simulations were also performed for control purposes and because of the high correlation between the behavior of $2A_{max}$ and averaged order parameter, only $2A_{max}$ results were included in the manuscript. In fact, EPR spectra are composed of several superimposed spectral components, since membrane is a heterogeneous structure composed of the regions with different fluidity characteristics. Therefore, except from direct spectral evaluation, EPR spectra of the studied samples were simulated by a computer program called EPRSIMC using a multi-component fast restricted wobbling motion approximation to obtain more precise description of the membrane characteristics. The model used for fitting procedure allows up to four spectral components with different spectral parameters [19,28]. The model parameters provided for each spectral component are the open cone angle ϑ of the wobbling motion, asymmetry angle of the cone φ, one effective rotational correlation time τ_c, additional broadening constant W, polarity correction factor p_a, proticity and the weight of each spectral component which describes the relative amount of the spin probes with particular motional mode.