Interaction of Antidepressant Drug, Clomipramine, with Model and Biological Stratum Corneum Membrane as Studied by Electron Paramagnetic Resonance

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ABSTRACT: The interactions of tricyclic antidepressant drug, clomipramine (CLO), with pig ear stratum corneum (SC) and model membranes were investigated by electron paramagnetic resonance (EPR) spin labeling to get some insight into the possible application of this drug in transdermal delivery. The changes in membrane characteristics caused by CLO in the regions that are close to the water–lipid interfaces and the central parts of the membranes were searched. The experimental results were supported by computer simulation of EPR spectra, which showed heterogeneity of the membranes composed of regions with different fluidity characteristics. CLO was effective in both parts of the layers, indicating intercalation of the drug into model membranes as well as into the pig ear SC. Introduction of various molar ratios of CLO caused a decrease in the order parameter and an increase in the rotational diffusion of nitroxide moiety in different membrane regions as well as an increase in the polarity of spin probe environment. It also changed the number of resolved spectral components, which reflects the heterogeneity of the membrane. The fluidizing effect of CLO on pig ear SC throughout the whole membrane layers indicates that CLO penetrates into the SC, which is important for its transdermal delivery. © 2013 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 102:3762–3772, 2013

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INTRODUCTION

Clomipramine (CLO), a tricyclic antidepressant (TCA), is a surface-active drug. TCAs, like other surface-active drugs (e.g., antibiotics, antihistamines, anesthetics) with the polar head group and hydrophobic tail, organize themselves in aqueous media in the form of micelles, bilayers, and monolayers. Aggregation or association depends on their geometry, hydrophilic group properties, and concentration.[1,2] Intercalation of the drugs into membranes leads to various effects in the membranes such as permeability and shape changes, lipid or drug flip-flop, domain deformation, and solubilization.[3] Different experimental techniques such as electron paramagnetic resonance (EPR), nuclear magnetic resonance (NMR), fluorescence, differential scanning calorimetry (DSC), X-ray and neutron diffraction, and so on are used to investigate the interaction between surface active drug and membrane. Previously, some studies were carried out on the interaction of TCAs with liposomes composed of different phospholipids by using radioligand binding method. Both surface adsorption and incorporation of TCA molecules into bilayer were proposed.[4] It was shown that the chlorinated TCAs (CLO and norclomipramine) bind to the membrane with twice the affinity of their nonchlorinated analogs (desipramine and imipramine).[5] The perturbations in the membrane caused by TCAs strongly depend on the chemical structure and effective charge of these drugs.[6] However, comparison of the results indicates that the interaction of TCA with membrane depends not only on the charge and structure of TCA but also on the composition of liposome membrane, pointing to a need to do further studies in this area.

A disadvantage of TCA drugs is that they may cause high gastrointestinal sensitivity in some patients after oral application. Therefore, it was suggested that transdermal delivery of TCAs could help to overcome this problem. It has recently been reported that it is necessary to overcome some problems like poor permeability of the drug through the stratum corneum (SC) and side effects on the skin after sun exposure resulting from transdermal delivery of drugs.[7] Hence, further investigation of membrane–TCA interaction should be performed on SC.

The SC is the uppermost layer of skin and it consists of keratin filled corneocytes and lipids. SC lipids are mainly composed of ceramides (CER), free fatty acids, and cholesterol (CH).[8,9] The majority of lipids form solid/crystalline lamellar structures with hexagonal or orthorhombic packing of lipids within the lamellae, which are responsible for barrier function of SC. These lipids coexist with fluid lipids organized in disordered liquid crystalline phase, which seems to be important for diffusional transport of molecules.[10,11] Because SC serves as the barrier of the skin, it has been studied extensively using different techniques. In recent studies, hydration of SC

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and its effects on phase transition, mobility, and transport of molecules were investigated using sorption microcalorimetry,[12] ATR-FTIR,[10] termomicroscopy, DSC,[13] wide-line ^{1}H NMR[12], ^{13}C solid-state NMR[11], and EPR.[14,15] It was found that the ratio between fluid and rigid lipids increases upon the hydration, what could be a reason for the enhanced permeability of intact SC upon hydration.[11–14]

For efficient topical delivery of TCA through the skin, it is important to know how TCA influences SC, and its structural and physical characteristics. EPR is one of the methods that can give some insight into tricyclic drugs–membrane interactions, its transport through the skin and side effects due to exposure to sun light. Interactions of antimicrobial agents,[16,17] neuroleptic drugs[18] or chemopreventive agents[19] with model and biological membranes and drug transport across the membranes or tissues[20,21] are some examples of such studies, proving how useful EPR spectroscopy could be in the investigation of membrane–drug interaction. We have recently performed an EPR study on the interaction of CLO, the member of TCA group, with liposomes of different composition. The role of CH and alkyl chain saturation in the interaction of CLO with the liposome membranes was investigated. In liposomes with saturated chains, CLO decreased the phase transition temperature, disordered the membrane and increased polarity in the upper part of the membrane. Presence of 30 mol % CH increased the fluidizing effect of CLO. Fewer effects were observed in liposomes with unsaturated chains[22] and no effect was observed in erythrocyte membrane (unpublished results).

Electron paramagnetic resonance with spin probes was also found as a sensitive method for characterization of protein and lipid fraction of SC. A spin-labeled maleimide derivative was used to evaluate SC protein mobility,[23,24] whereas for monitoring the properties of SC lipids, the stearic acid spin probes were used[14,15,25–29] and was supported with the EPR spectra line shape simulation.[26,28,30] The results give information about the alkyl chain packing and mobility in the SC membranes. The method was found to be sensitive to the changes produced due to the hydration of the skin,[14] different surfactants,[15] permeation enhancers,[30] drugs,[29] and was shown to be useful in the investigations of protein–lipid interaction in native SC,[31] as well as in different cases of skin diseases and lesions.[32]

In this study, EPR was used to search interaction of surface-active antidepressant drugs with SC isolated from pig ear skin and with a model membrane (liposomes), which mimics the composition of SC. As a TCA drug, CLO—known to bind to the membrane with twice the affinity of its nonchlorinated analogs imipramine[5]—was used. As far as we know, CLO has not been used in similar studies yet. Isolated SC was found to have the same fluidity characteristic as SC obtained by stripping procedure *in vivo*[27] but was more sensitive to the influence of different surfactants.[15] Therefore, in our study, we used SC from pig ear skin obtained by isolation procedure.

MATERIALS AND METHODS

Materials

Bovine brain CER (Type III, 98% purity) from Fluka (Sigma-Aldrich Co. LLC., Buchs, Switzerland), CLO (98% purity), CH (99% purity), chloroform (99%–99.4% purity), palmitic acid (99% purity), were all obtained from Sigma, phosphate-buffered saline (without Ca,Mg) was obtained from Dr. Zeydanlı (Dr. Zeydanlı Life Sciences Ltd. Şti., Ankara, Turkey). Spin labels: 5- and 16-doxyl stearic acids (5-DS, 16-DS) were obtained from Aldrich.

Preparation of Model Membranes (CER/CH liposomes)

As a model, liposomes with lipid composition—50 wt % bovine brain CER, 30 wt % CH, and 20 wt % palmitic acid—which mimic lipid composition of SC membrane[8] were prepared. For EPR measurements, lipophilic spin labels 5-doxyl stearic acid (5-DS) with nitroxide moiety on the 5th C atom of the alkyl chain counting from the carboxylic acid group and 16-doxyl stearic acid (16-DS) with nitroxide group on the 16th C atom were chosen. 5-DS monitors membrane characteristics in the interfacial regions of membrane layers, whereas 16-DS monitors the characteristics close to the central part of the bilayers. Spin-labeled liposome dispersions were prepared by solving lipids, spin labels (5-DS or 16-DS), and 0, 1, 5, or 10 wt % of CLO in chloroform, (1.3, 6.5, and 13 mol %, respectively, taking into account an estimated molecular weight of SC lipids 785 g/mol[33]). Chloroform was evaporated first with a stream of nitrogen gas and after that samples were kept to remove residual chloroform at low pressure for overnight. Dry films were hydrated with phosphate buffer, vortexed above $T = 353$ K and then centrifuged (Eppendorf 5804-R; Eppendorf-Nethelar-Hinz GmbH, Hamburg, Germany) at 10,000 rpm (at 292 K) for 30 min. Total amount of lipids without CLO was 66.7 g/L, and molecular ratio between lipids and spin label was 0.01. Measurements were completed within 2 days after sample preparation.

Electron paramagnetic resonance measurements were performed with modulation frequency 100 kHz, incident microwave power 10 mW and modulation amplitude 0.2 mT on a Bruker EMX spectrometer (Bruker Biospin GmbH, Germany) in temperature range of 283–328 K with ER4103TM cylindrical cavity. Sample temperature was controlled by a Bruker VT4111 temperature controller to ± 1 K.

Preparation of Pig Ear SC Membranes

Stratum corneum was isolated from pig ear skin following the procedure described previously.[27] In short, pig ear skin taken from the outer side of the porcine auricle was excised, and a very thin skin slice was cutoff. To separate epidermis from dermis, the skin slice was put in water at 333 K for 2 min, followed by mechanical removal. The epidermis was placed on a filter paper (SC side up) immersed into 0.2% trypsin for 24 h at 310 K to separate SC. Any softened epidermis was removed by mild agitation of SC sheet and the thickness of SC was measured by microscope and was found to be approximately 20–30 μm thick. SC samples were kept in humid atmosphere in the refrigerator (277 K).

For spin labeling, five to six layers of SC (1–3 mm width, 3–5 mm long and cca 150 μm thick) were incubated in 5 mL of 5-DS or 16-DS spin probe solution in water (26 μM) in presence or absence of CLO (0.01–19 mM) for 1 h at 310 K. Then, the SC was well washed in water so that unattached spin probe could be removed. Afterward, it was placed onto the flat surface of a home-made narrow Teflon tissue cell for EPR measurements. Experiments were repeated using fully hydrated SC samples obtained from four different pigs.

Experiments were performed on Bruker ELEXSYS E500 EPR X-band spectrometer (Bruker Biospin GmbH, Germany) in