Biophysical Chemistry 135 (2008) 7–13

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

Human cells and cell membrane molecular models are affected in vitro by chlorpromazine

Mario Suwalsky [a,*], Fernando Villena [b], Carlos P. Sotomayor [c], Silvia Bolognin [d], Paolo Zatta [d]

[a] Faculty of Chemical Sciences, University of Concepción, Concepción, Chile
[b] Faculty of Biological Sciences, University of Concepción, Concepción, Chile
[c] Institute of Chemistry, Catholic University of Valparaíso, Valparaíso, Chile
[d] CNR-Centre of Metalloproteins, University of Padova, Padova, Italy

ARTICLE INFO

Article history:
Received 14 January 2008
Received in revised form 28 February 2008
Accepted 28 February 2008
Available online 6 March 2008

Keywords:
Chlorpromazine
Human erythrocytes
Neuroblastoma cells
Lipid bilayer

ABSTRACT

This study presents evidence that chlorpromazine (CPZ) affects human cells and cell membrane molecular models. Human SH-SY5Y neuroblastoma cells incubated with 0.1 mM CPZ suffered a decrease of cell viability. On the other hand, phase contrast microscopy observations of human erythrocytes indicated that they underwent a morphological alteration as 1 μM CPZ changed their discoid normal shape to stomatocytes, and to hemolysis with 1 mM CPZ. X-ray diffraction experiments performed on dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylethanolamine (DMPE) bilayers, classes of the major phospholipids present in the outer and inner sides of the erythrocyte membrane, respectively showed that CPZ disordered the polar head and acyl chain regions of both DMPC and DMPE, where these interactions were stronger with DMPC bilayers. Fluorescence spectroscopy on DMPC LUV at 18 °C confirmed these results. In fact, the assays showed that CPZ induced a significant reduction of their generalized polarization (GP) and anisotropy (r) values, indicative of enhanced disorder at the polar head and acyl chain regions of the DMPC lipid bilayer.

1. Introduction

Schizophrenia, a syndrome of unknown etiology characterized by delusions, hallucinations, and disturbance of thought, is one of the most pervasive and socially disabling of all psychiatric disorders [1]. Several hypotheses have been raised on the cause of this serious disease; however there is little consensus over the years on the clinical definition of this disorder, or if it consists of one or many disorders with common clusters of symptoms. Stanley et al. [2] observed a reduction in precursors of membrane phospholipids during both the early and chronic stages of the illness, and concomitantly an increase of the breakdown products of membrane phospholipids at the early stage of the schizophrenia. Several other authors report alteration in membrane and phosphorus metabolism in schizophrenia [3,4].

Chlorpromazine (CPZ) is a neuroleptic drug of the phenothiazin family, and is widely used in the treatment of schizophrenia. Its mode of action at the cellular level remains elusive; hence, much effort has been aimed to characterize its interaction with various membrane systems [5,6]. CPZ binds significantly to cell membranes [7]; its amphipathic character enables it to partition into the lipid bilayer, reaching in this way the central nervous system [8]. Since the tricyclic ring of CPZ is hydrophobic (Fig. 1), it might easily partition into the bulk hydrocarbon phase of membrane bilayer, while its hydrophilic propylamine tail can interact with the bilayer head group regions [9]. While many reports have discussed the importance of hydrophobic and electrostatic forces in the interaction of CPZ with membrane bilayers, detailed characterization of these forces and their role in drug-membrane association is lacking. Further, it is unclear whether CPZ electrostatic interactions with the membrane are dictated more by the presence of the anionic lipid phosphatidylserine or the polyphosphoinositide lipids [9]. Changes in membrane fluidity are known to be associated to alterations in physiological processes of cell membranes such as carrier-mediated transport, enzyme activities, receptor binding, phagocytosis, endocytosis, cytotoxicity, and cell growth [10]. Moreover, membrane fluidity appears to change during cultured cell drug therapy [11]. Research on the mechanism of CPZ pharmacological action has been almost reduced to its blocking of dopaminergic receptors. However, CPZ also exerts effects upon muscarinic and α_1-adrenergic receptors, a possible reason for the many side effects associated with this treatment [9]. Since receptors are embedded in membranes, several authors have advised that changes in lipid fluidity may modify CPZ binding to receptors [12]. Recently, it has been demonstrated that CPZ was able to alter the integrity of most membrane organelles within a cell and that it also activated important modification in gene expression, followed by

* Corresponding author. Tel.: +56 41 2204171; fax: + 56 41 2245974.
E-mail address: msuwalsk@udec.cl (M. Suwalsky).

Abbreviations: CPZ, chlorpromazine; DMPC, dimyristoylphosphatidylcholine; DMPE, dimyristoylphosphatidylcholine; LUV, large unilamellar vesicles; r, fluorescence anisotropy; GP, generalized polarization; DPH, 1,6-diphenyl-1,3,5-hexatriene; laurdan, 6-dodecanoyl-2-dimethylaminonaphtalene; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PCM, phase contrast microscopy.

0301-4622/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bpc.2008.02.014

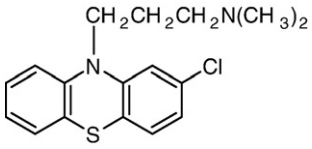

Fig. 1. Structural formula of chlorpromazine.

inhibition of protein synthesis [13]. Moreover, Sabaté et al. [14] estimated the risk of acute liver injury associated with the use of a series of drugs and they found a high incidence of acute liver injury among the population exposed to CPZ in comparison with the unexposed population.

In the course of in vitro systems search for the toxicity screening of biologically relevant chemicals, different cellular models have been applied to examine their adverse effects. The cell membrane is a diffusion barrier that protects the cell interior, and thus its structure and functions are susceptible to alterations as a consequence of interactions with chemical species. With the aim to better understand the molecular mechanisms of CPZ interaction with cell membranes, we have utilized human erythrocytes and molecular models of cell membranes. Human erythrocytes were chosen because having only one membrane and no internal organelles, it is an ideal cell system for studying basic drug–biomembrane interactions [5]. Additionally, although less specialized than many other cell membranes, they carry on enough similar functions, such as active and passive transport and the production of ionic and electric gradients, to be considered representative of the plasma membrane in general. The molecular models consisted of dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylethanolamine (DMPE) bilayers, representative of phospholipid classes located in the outer and inner monolayers of cell membranes, particularly of the human erythrocyte, respectively [15,16] and large unilamellar vesicles (LUV) of DMPC. CPZ capacity to perturb the multi-bilayer structures of DMPC and DMPE was evaluated by X-ray diffraction, DMPC LUV were studied by fluorescence spectroscopy, and human erythrocytes were observed by phase contrast microscopy. These systems and techniques have been used in our laboratories to determine the interaction with and the membrane-perturbing effects of other drugs that interact with the central nervous system [17,18]. Additional experiments were performed in human neuroblastoma cells SH-SY5Y in order to test the toxicity after treatments with CPZ. The MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay was used to explore the cell proliferation rate and cell viability. In the reaction, reductases of metabolically active, viable cells reduce the soluble MTT to the water-insoluble MTT-formazan which is spectrophotometrically detected.

2. Materials and methods

2.1. X-ray diffraction studies of DMPC and DMPE multilayers

CPZ capacity to perturb the structures of DMPC and DMPE multilayers was evaluated by X-ray diffraction. Synthetic DMPC (lot 80H-8371, MW 677.9), DMPE (lot 084 K-1676, MW 635.9), and chlorpromazine·HCl (C8138, MW 355.3) from Sigma (St. Louis, MO) were used without further purification. About 2 mg of each phospholipid was introduced into 1.5 mm diameter special glass capillaries, which were then filled with 200 ml of (a) distilled water and (b) aqueous CPZ solutions in a range of concentrations (0.05 mM to 5 mM). The specimens were X-ray diffracted after 1 h incubation at 37 °C and 60 °C with DMPC and DMPE, respectively. Specimen-to-film distances were 8 and 14 cm, standardized by sprinkling calcite powder on the capillary surface. Ni-filtered CuKα radiation from a Bruker Kristalloflex 760 (Karlsruhe, Germany) X-ray generator was used. The relative reflection intensities were obtained in an MBraun PSD-50M linear position-sensitive detector system (Garching, Germany); no correction factors were applied. The experiments were performed at 18±1 °C, which is below the main phase transition temperature of both DMPC and DMPE. Higher temperatures would have induced transitions to more fluid phases making the detection of structural changes harder. Each experiment was performed in triplicate and in case of doubts additional experiments were carried out.

2.2. Fluorescence measurements of DMPC large unilamellar vesicles (LUV)

CPZ influence on the physical properties of DMPC LUV was examined by fluorescence spectroscopy using DPH (1,6-diphenyl-1,3,5-hexatriene) and laurdan (6-dodecanoyl-2-dimethylaminonaphtalene) fluorescent probes (Molecular Probe, Eugene, OR). DPH is widely used as a probe for the hydrophobic regions of the phospholipid bilayers because of its favorable spectral properties. Its steady-state fluorescence anisotropy measurements were used to investigate the structural properties of DMPC LUV because it provides a measure of the hindered rotational diffusion of the fluorophore, restricted within a certain region such as a cone, due to the lipid acyl chain packing order. Laurdan, an amphiphilic probe, has a high sensitivity of excitation and emission spectra to the membranes' physical state. With the fluorescent moiety within a shallow position in the bilayer, laurdan fluorescence spectral shifts provide information of its molecular dynamic properties at the level of the phospholipid polar headgroups. The laurdan fluorescence shifts were quantified using the generalized polarization (GP) concept [19], which reflects the solvatochromic properties of the reporter group.

DMPC LUV suspended in water were prepared by extrusion of frozen and thawed multilamellar liposome suspensions (final lipid concentration 0.4 mM) through two stacked polycarbonate filters of 400 nm pore size (Nucleopore, Corning Costar Corp., MA) under nitrogen pressure at 37 °C, which is above the lipid phase transition temperature. DPH and laurdan were incorporated into DMPC LUV by addition of 2 μl/ml aliquots of 0.5 mM solutions of the probe in dimethylformamide and ethanol respectively in order to obtain final analytical concentrations of 0.5 μM, incubating them at 37 °C for 45 min. Fluorescence spectra and anisotropy measurements were performed in a phase shift and modulation K_2 steady-state and time-resolved spectrofluorometer (ISS Inc., Champaign, IL) interfaced to computer. Software from ISS was used for data collection and analysis. Measurements of LUV suspensions were made at 18 °C and 37 °C using 10 mm path-length square quartz cuvettes. Sample temperature was controlled by an external bath circulator (Cole-Parmer, Chicago, IL) and monitored before and after each measurement using an Omega digital thermometer (Omega Engineering Inc., Stanford, CT). Anisotropy measurements were made in the L configuration using Glan Thompson prism polarizers (I.S.S.) in both exciting and emitting beams. The excitation wavelength was set at 360 nm, and the emission was measured using a WG-420 Schott high-pass filter (Schott WG-420, Mainz, Germany) with negligible fluorescence. DPH fluorescence anisotropy (r) was calculated according to the definition: $r=(I_{||}-I_{\perp})/(I_{||}+2I_{\perp})$ where $I_{||}$ and $I_{\perp}$ are the corresponding vertical and horizontal emission fluorescence intensities with respect to the vertically polarized excitation light [20]. Laurdan fluorescence spectral shifts were quantitatively evaluated using the GP concept (see above), which is defined by the expression $GP=(I_b-I_r)/(I_b+I_r)$, where I_b and I_r are the emission intensities at the blue and red edges of the emission spectrum, respectively. These intensities have been measured at the emission wavelengths of 440 and 490 nm, which correspond to the emission maxima of laurdan in the gel and liquid-crystalline phases, respectively [21]. CPZ was incorporated in LUV by addition of adequate (0.1 M) aliquots of CPZ solution in order to obtain the different concentrations used in this work. The samples thus prepared were then incubated at 37 °C for ca. 15 min and measured at 18 and 37 °C; at 18 °C because the X-ray experiments were performed at about this

temperature, and at 37 °C because that is the normal temperature at which erythrocytes circulate in humans, temperature at which were also incubated for the phase contrast microscopy observations. Blank subtraction was performed in all measurements using unlabeled samples without probes. The data presented in Figs. 1 and 2 represent mean values and standard error of ten measurements in two independent samples. Unpaired Student's *t*-test was used for statistical calculations.

2.3. Phase contrast microscopy (PCM) studies of human erythrocytes

In vitro interaction of CPZ with erythrocytes was attained by incubating it with red blood cell suspensions derived from healthy human male donors not receiving any pharmacological treatment. Drops of blood obtained by ear lobe puncture were collected in a plastic tube containing 1 ml of saline (0.9% NaCl). To dilute the erythrocytes and to prepare different concentration samples, this blood suspension was transferred into a syringe with 10 ml of saline. From this stock suspension, the following were prepared in saline: a) control (stock suspension without CPZ) and b) suspensions containing CPZ in a range of concentrations (5 μM, 1 mM, 5 mM and 10 mM). The control and the samples with CPZ were incubated at 37 °C for 1 h and then, studied in a phase contrast microscope.

2.4. Viability studies on human neuroblastoma cells SH-SY5Y

SH-SY5Y human neuroblastoma cells were purchased from ECACC (European Collection of Cell Culture, Salisbury, UK). SH-SY5Y were cultured in DMEM/ F12 (Gibco, Carlsbad, CA) medium containing 15% (v/v) fetal bovine serum (FBS, Sigma Aldrich, St. Louis, MO), 100 units/ml penicillin (Gibco, Carlsbad, CA) and streptomycin (100 μg/ml; Gibco, Carlsbad, CA), at 37 °C with 5% CO_2 in a humidified atmosphere (90% humidity). The medium was replaced every 2 days. 0.25% Trypsin–EDTA solution and phosphate buffered saline (PBS) were obtained from Sigma Aldrich (St. Louis, MO). Cell viability was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma Aldrich, St. Louis, MO) reduction assay. Briefly, SHSY5Y cells were seeded into 96-well plates (at a density of 8×10^4 cells per well, in 100 μl medium). 2% FBS medium containing CPZ at a concentration range from 1 nm to 1 mM was added to the cells for 1, 6, 24 and 48 h. CPZ was prepared as a 50 mM stock solution in EtOH, the final concentration of the solvent in the well was 1.6%. MTT (5 mg/ml) was added to each well and incubated in the dark at 37 °C for 3 h followed by cells lysis and spectrophotometric measurement at 550 nm (Microplate SPECTRAmax^R at 550). The MTT solution was carefully decanted off, and formazan was extracted from the cells with 100 μl of acidic isopropanol (0.04 M HCl in absolute isopropanol) in each well [22]. Color was measured with a 96-well ELISA plate reader. All MTT assays were performed in triplicate. All readings were compared with the control, which represented 100% viability. Data regarding MTT assay were analyzed using the *T*-test and Primer software, and values were reported as highly statistically significant if $P<0.01$. Results are presented as mean±standard deviation.

3. Results

3.1. X-ray diffraction studies of DMPC and DMPE multilayers

Fig. 2A exhibits the results obtained by incubating DMPC with water and CPZ. As expected, water altered the structure of DMPC, as its bilayer repeat (phospholipid bilayer width plus the layer of water) increased from about 55 Å in its dry crystalline form [23] to 64 Å when immersed in water, and its low-angle reflections (indicated as LA), which correspond to DMPC polar terminal groups, were reduced to only the first two orders of the bilayer repeat. On the other hand, only one strong reflection of 4.2 Å showed up in the wide-angle region (indicated as WA), which corresponds to the average distance

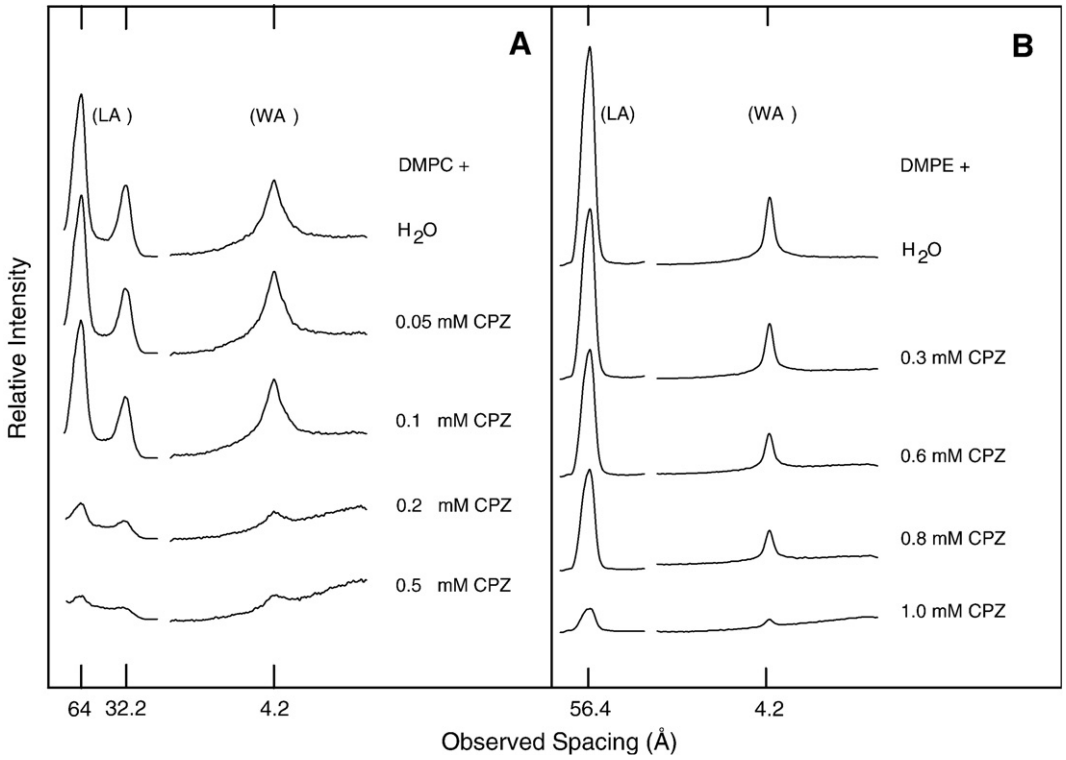

Fig. 2. Microdensitograms from X-ray diffraction patterns of (A) DMPC and (B) DMPE in water and aqueous solutions of chlorpromazine hydrochloride (CPZ); (LA) and (WA) correspond to low- and wide-angle reflections, respectively.

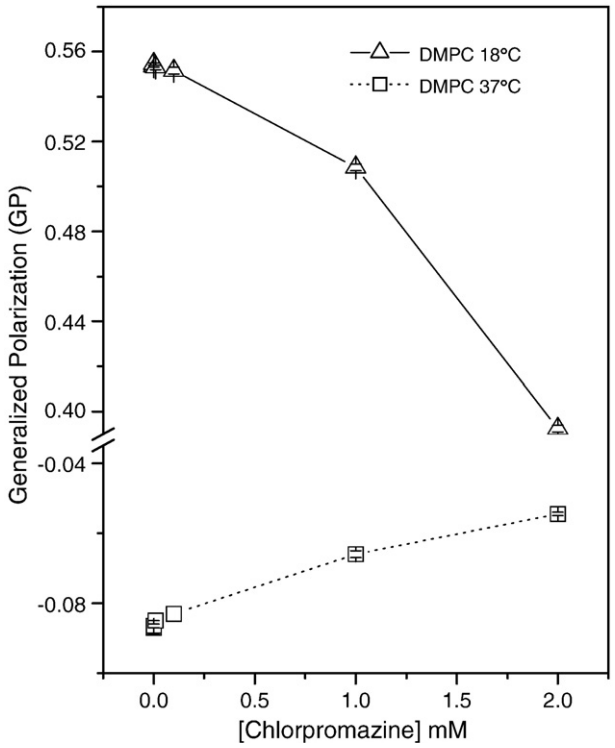

Fig. 3. Effect of chlorpromazine hydrochloride (CPZ) on the generalized polarization (GP) of DMPC LUV at 18 °C and 37 °C.

between fully extended acyl chains organized with rotational disorder in hexagonal packing. These results were indicative of the gel state reached by DMPC bilayers. Fig. 2A discloses that after exposure to 0.1 mM CPZ, there was a slight weakening of DMPC reflection intensities. These considerably decreased with 0.2 mM CPZ (equivalent to CPZ:DMPC 1:75 molar ratio), and became almost negligible with 0.5 mM, an effect observed in both the low- and wide-angle DMPC reflections. From these results, it can be concluded that CPZ

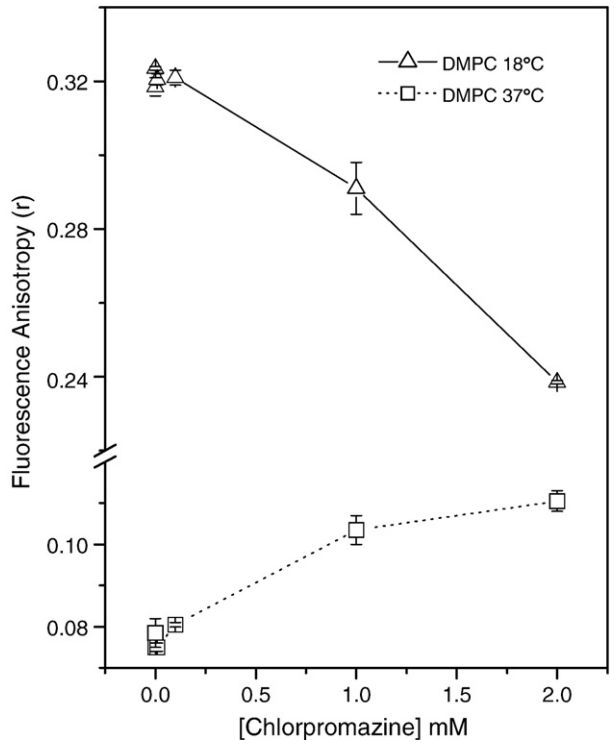

Fig. 4. Effect of chlorpromazine hydrochloride (CPZ) on the fluorescence anisotropy (*r*) of DMPC LUV at 18 °C and 37 °C.

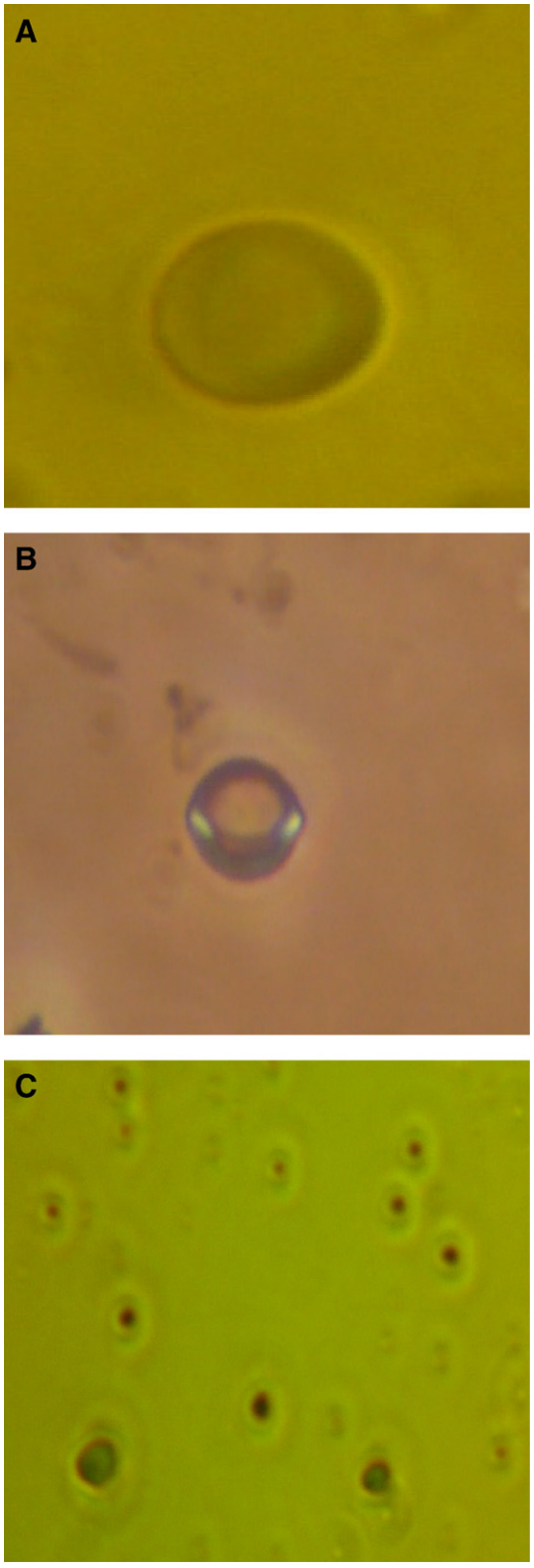

Fig. 5. Phase contrast microscopy (PCM) observations of human erythrocytes incubated with chlorpromazine hydrochloride (CPZ), X 1920. (A) control; (B) 5 μM CPZ (C) 1 mM CPZ.

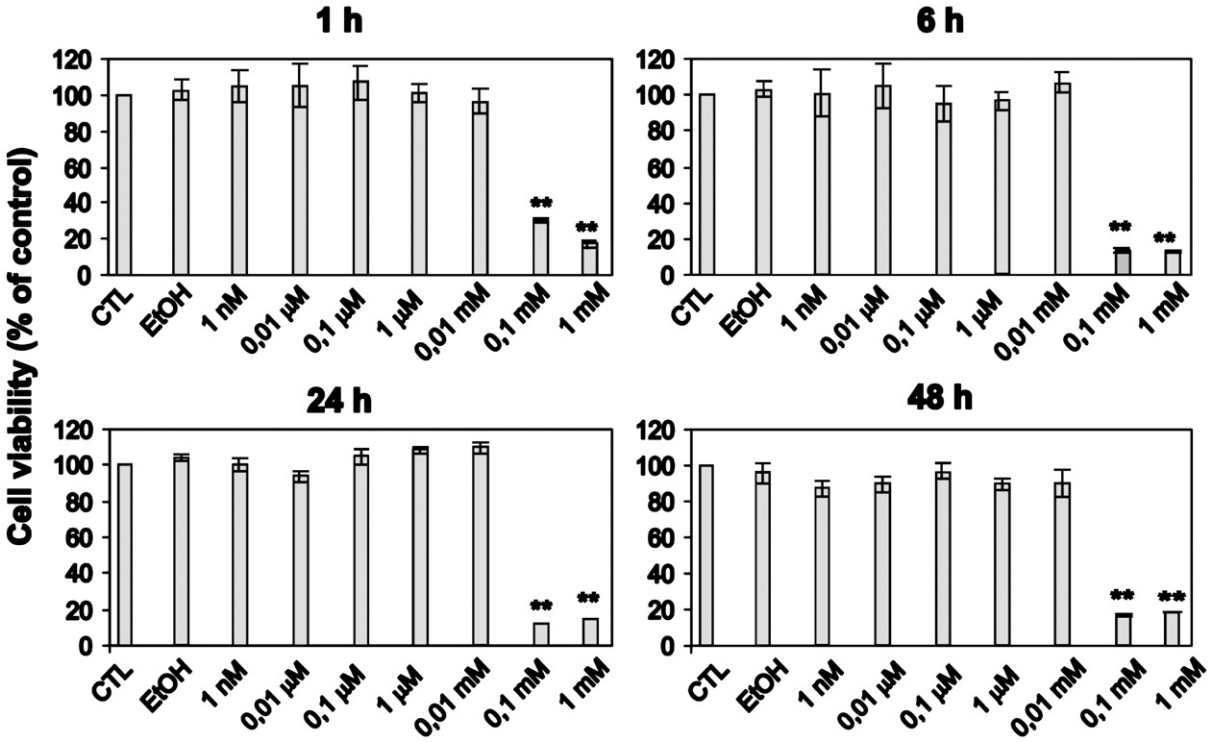

Fig. 6. Cytotoxicity assay in SH-SY5Y cells after treatment with CPZ in a range of concentrations between 1 nM and 1 mM. Neuroblastoma redox activity was measured by MTT assay. The data represented are mean ± SD of three individual experiments, each done in triplicate. ** $P<0.01$ vs control.

produced a significant structural perturbation, affecting both the polar head and acyl chain regions of DMPC bilayers and, as a consequence, a disruption of the in-plane structure and the bilayer stacking. Results from similar experiments with DMPE are presented in Fig. 2B. As reported elsewhere, water did not significantly affect the bilayer structure of DMPE [23]. It can be observed that increasing CPZ concentrations gradually but slightly reduced DMPE reflection intensities; however all of them were still present with 0.8 mM CPZ, and were considerably affected only at the highest CPZ concentration assayed (1.0 mM).

3.2. Fluorescence measurements of DMPC large unilamellar vesicles (LUV)

CPZ concentration-dependent effects on DMPC LUV were explored at two different depths of the lipid bilayer: (1) at the hydrophilic/hydrophobic interface level, estimated from the laurdan fluorescence spectral shift through the general polarization (GP) parameter, and (2) in the deep hydrophobic core, determined by the DPH steady-state fluorescence anisotropy (r). Fig. 3 shows that CPZ incorporation from 1 μM to 2 mM to DMPC LUV induced a sharp decrease in general polarization at 18 °C. This result can be interpreted as a disordering effect induced by CPZ on DMPC polar head groups when the bilayer is in the gel state. In contrast, similar measurements performed at 37 °C, when the bilayer is in the much more fluid liquid-crystalline state, showed that there was a mild increase in GP, implying a decrease in polarity and/or molecular dynamics, presumably due to a moderate ordering of DMPC polar head groups. Fig. 4 shows that at 18 °C CPZ also induced a sharp decrease of the anisotropy at concentrations higher than 0.1 mM, a result that implies a structural perturbation of the DMPC LUV acyl chain region in the gel state; however, a mild increase of the anisotropy was observed when the same experiment was performed at 37 °C, implying a partial ordering effect in the hydrocarbon chain region of the lipid bilayer in the liquid-crystalline state.

3.3. Phase contrast microscopy (PCM) studies of human erythrocytes

Observations by PCM of red cells incubated with 5 μM CPZ revealed abnormalities in their shapes. In fact, in contrast to the normal discoid erythrocyte profile (Fig. 5A), about a fourth of chlorpromazine treated cells underwent a stomatocytic or cup-shape type of alteration, i.e., developed invagination of one surface and evagination of the opposite face (Fig. 5B). When increasing CPZ concentrations above 1 mM, the drug caused total hemolysis of the erythrocyte treated samples (Fig. 5C).

3.4. Viability studies on human neuroblastoma cells SH-SY5Y

The effects of CPZ in a concentration range from 1 nM to 1 mM on human SH-SY5Y neuroblastoma cells were also assayed. With this aim, cells were incubated for 1, 6, 24 and 48 h. As can be observed in Fig. 6, the greater decrease of cell viability was observed with 0.1 mM and 1 mM treatments. This toxic effect was immediate since it was evident only after 1 h, and it was not recovered because it remained after 48 h. The other concentrations tested were unable to produce any significant effect after 1, 6, 24, and 48 h. A somewhat lower decrease of viability was observed after 48 h with 0.01 mM CPZ, although it was not significant. The presence of the solvent alone did not alter the cellular redox activity with respect to the control. These findings clearly showed that from 0.01 mM and 0.1 mM, the threshold lies between the toxic and non-toxic CPZ concentration.

4. Discussion

This study presents the following evidence that CPZ affects human cells and molecular models of the cell membrane. In the viability studies, CPZ showed significant toxicity in the 0.1–1 mM concentration range. Recent works indicate that CPZ's toxic effects could be ascribed to its interaction with anionic lipids [9]. De Filippi et al. [13] showed that CPZ diffused within the cells and altered membranes of

intracellular organelles. The authors proposed that CPZ inhibited intracellular trafficking by modifying the structure and the net changes of membranes on secretary/endocytic compartments as well as strongly inhibited protein synthesis in mammalian cells. It has also been shown that CPZ was antagonized by calmodulin when added to fibroblast cell cultures, giving rise to a time- and dose-dependent decrease of sphingomyelinase activity [24]. Moreover, CPZ inhibited Ca^{2+} flux across cellular membranes [25] and was also able to inhibit the Ca^{2+}–calmodulin interaction [26]. These findings taken together may account for the wide toxicity shown on neuroblastoma cells with higher CPZ concentrations (0.1–1 mM). The toxic effect due to CPZ treatment was also observed on erythrocytes in the same concentration range. In fact, phase contrast microscopy observations of human erythrocytes indicated that they underwent a morphological alteration as their discoid normal shape changed to stomatocytes and to hemolysis with 1 μM CPZ and 1 mM CPZ, respectively. This latter value is very close to the 0.8 mM reported in the literature [27]. The mechanism of CPZ-induced shape changes in erythrocytes has long been under study. According to the bilayer-couple hypothesis [28,29], the morphological changes induced in erythrocytes by foreign molecules are due to the differential expansion of their two monolayers. Thus, speculated shapes (echinocytes) are induced when the added compound is inserted in the outer monolayer, whereas cup shapes (stomatocytes) arise when the compound accumulates in the inner monolayer. The X-ray diffraction experiments performed on bilayers made up of DMPC and DMPE, classes of the major phospholipids present in the outer and inner sides of the erythrocyte membrane, respectively showed that CPZ disordered the polar head and acyl chain regions of both DMPC and DMPE, where these interactions are stronger with DMPC bilayers. DMPC and DMPE differ only in their terminal amino groups, these being $^{+}N(CH_3)_3$ in DMPC and $^{+}NH_3$ in DMPE. Moreover, both molecular conformations are very similar in their dry crystalline phases [23] with the hydrocarbon chains mostly parallel and extended, and the polar head groups lying perpendicularly to them. However, the gradual hydration of DMPC results in water filling the highly polar interbilayer spaces with the resulting width increase. This phenomenon allows the incorporation of CPZ into DMPC bilayers; the drug cationic tertiary propylamine chain most likely interacts with the negatively charged phosphates of DMPC polar head groups while the hydrophobic tricyclic ring partitions into DMPC acylic chains with the resulting disruption of DMPC bilayer structure. On the other hand, DMPE molecules pack tighter than those of DMPC due to their smaller polar groups and higher effective charge, resulting in a very stable bilayer system that is not significantly affected by water. This organization does not prevent CPZ from interacting with DMPE and perturbing its structure, although these effects were much milder than those observed in DMPC. Fluorescence spectroscopy on DMPC LUV at 18 °C confirmed these results. In fact, the assays showed that CPZ induced a significant reduction of their GP and *r* values, indicative of enhanced disorder at the polar head and acyl chain regions of DMPC lipid bilayer.

According to the bilayer-couple hypothesis, the preferential interaction of CPZ with DMPC, a class of lipid mainly located in the outer monolayer of the erythrocyte membrane, should have induced echinocytosis instead of the observed stomatocytosis. One explanation for this discrepancy could be based on the lipid scrambling mechanism proposed by Schrier et al. [30]. Accordingly to it, some cationic amphipaths produce a rapid scrambling of the erythrocyte bilayer with phosphatidylcholines (PC) and sphyngomyelins (SM) moving inward while phosphatidylethanolamines (PE) move outward along with phosphatidylserines (PS). Thus, the interaction of CPZ with PC in the inner monolayer would lead to stomatocytosis, an effect that can be produced by as little as 0.6% enrichment of the cytoplasmic monolayer [30]. The mechanism of CPZ-induced stomatocytosis in erythrocytes has long been under study. While lipid scrambling offers an explanation, it appears that selective inner monolayer intercalation of the drug also provides an explanation for the morphological effect [5]. In fact, there are reports that CPZ interacts with phosphatidylserine bilayers [31,32], as well as with polyphosphoinositoside lipids [9], while other studies indicate that CPZ interacts with proteins such as spectrin [33].

Amazingly, the therapeutic range for plasma CPZ concentrations and the relationship of plasma concentration to clinical response and toxicity have not been clearly established [7,34]. Nevertheless, the experimental findings are certainly of interest as they indicate that a CPZ concentration as low as 1 μM affects the human erythrocyte shape. It must be considered that alteration of the normal biconcave shape of red blood cells increases their resistance to entry into capillaries, which could contribute to decreased blood flow, loss of oxygen, and tissue damage through microvascular occlusion [35,36].

Acknowledgements

The authors thank Fernando Neira for his technical assistance. This work was supported by FONDECYT grants 1060990 and 7070130.

References

[1] L.E. De Lisi, R.J. Wyatt, Neurochemical aspects of schizophrenia, in: A. Lajtha (Ed.), Handbook of Neurochemistry, Pathological Neurochemistry, vol. 10, Plenum Press, New York, 1985, pp. 553–587.
[2] J.A. Stanley, P.C. Williamson, D.J. Drost, T.J. Carr, R.J. Rylett, A. Malla, R.T. Thompson, An in vivo study of the prefrontal cortex of schizophrenic patients at different stages of illness via phosphorus magnetic resonance spectroscopy, Arch. Gen. Psychiatry. 52 (1995) 399–406.
[3] J.W. Pettegrew, M.S. Keshavan, K. Panchalingam, S. Strychor, D.B. Kaplan, M.G. Tretta, M. Allen, Alterations in brain high energy phosphate and membrane phospholipids metabolism in first-episode, drug-naive schizophrenics. A pilot study of the dorsal prefrontal cortex by in vivo phosphorus 31 nuclear magnetic resonance spectroscopy, Arch. Gen. Psychiatry. 48 (1991) 563–568.
[4] H. Fukuzako, T. Fukuzako, T. Hashiguchi, S. Kodama, M. Takigawa, T. Fujimoto, Changes in levels of phosphorus metabolites in temporal lobes of drug-naive schizophrenic patients, Am. J. Psychiatr. 156 (1999) 1205–1208.
[5] J.Y. Chen, W.H. Huestis, Role of membrane lipid distribution in chlorpromazine-induced shape change of human erythrocytes, Biochim. Biophys. Acta 1323 (1997) 299–309.
[6] N. Maruoka, T. Murata, N. Omata, Y. Takashima, H. Tanii, Y. Yonekura, Y. Fujibayayashi, Y. Wada, Effects of chlorpromazine on plasma membrane permeability and fluidity in the rat brain: a dynamic positron autoradiography and fluorescence polarization study, Prog. Neuropsychopharmacol. Biol. Psychiatry 31 (2007) 178–186.
[7] R.J. Baldessarini, F.I. Tarazi, Pharmacotherapy of Psychosis, in: L.L. Brunton (Ed.), Goodman & Gilman's The Pharmacological Basis of Therapeutics, 11th ed., McGraw-Hill, USA, 2006, p. 475.
[8] A. Wisniewska, A. Wolnicka-Glubisz, ESR studies on the effect of cholesterol on chlorpromazine interaction with saturated and unsaturated liposome membranes, Biophys. Chemist. 111 (2004) 43–52.
[9] J.Y. Chen, L.S. Brunauer, F.C. Chu, C.M. Helsel, M.M. Gedde, W.H. Huestis, Selective amphipathic nature of chlorpromazine binding to plasma membrane bilayers, Biochim. Biophys. Acta 1616 (2003) 95–105.
[10] A. Spector, M.A. Yorek, Membrane lipid composition and cellular functions, J. Lipid Res. 26 (1985) 1015–1035.
[11] H. Toplak, R. Zuehlke, S. Loidl, A. Hermetter, U.E. Honegger, U.N. Wiesmann, Single and multiple desipramine exposure of cultured cells, Biochem. Pharmacol. 39 (1990) 1437–1443.
[12] H.-O. Jang, D.-K. Jeong, S.-H. Ahn, Ch.-D. Yoon, S.-Ch. Jeong, S.-D. Jin, I. Yun, Effects of chlorpromazine.HCl on the structural parameters of bovine brain membranes, J. Biochem. Mol. Biol. 37 (2004) 603–611.
[13] L. De Filippi, M. Fournier, E. Cameroni, P. Linder, C. De Virgilio, M. Foti, O. Deloche, Membrane stress is coupled to a rapid translational control of gene expression in chlorpromazine-treated cells, Curr. Genet. 52 (2007) 171–185.
[14] M. Sabaté, L. Ibáñez, E. Pérez, X. Vidal, M. Buti, X. Xiol, A. Mas, C. Guarner, M. Forné, R. Solá, J. Castellote, J. Rigau, J.R. Laporte, Risk of acute liver injury associated with the use of drugs: a multicentre population survey, Aliment. Pharmacol. Ther. 25 (2007) 1401–1409.
[15] J.M. Boon, B.D. Smith, Chemical control of phospholipid distribution across bilayer membranes, Med. Res. Rev. 22 (2000) 251–281.
[16] P.F. Devaux, A. Zachowsky, Maintenance and consequences of membrane phospholipids asymmetry, Chem. Phys. Lipids 73 (1994) 107–120.
[17] M. Suwalsky, S. Mennickent, B. Norris, F. Villena, C.P. Sotomayor, Effects of the antiepileptic drug carbamazepine on human erythrocytes, Toxicol. In Vitro 20 (2006) 1363–1369.
[18] M. Suwalsky, S. Mennickent, B. Norris, F. Villena, F. Cuevas, C.P. Sotomayor, The antiepileptic drug diphenylhydantoin affects the structure of the human erythrocyte membrane, Z. Naturforsch. 59c (2004) 427–431.

[19] T. Parasassi, E. Gratton, Membrane lipid domains and dynamics as detected by laurdan fluorescence, J. Fluoresc. 5 (1995) 59–69.
[20] J.R. Lakowicz, Principles of Fluorescence Spectroscopy, Plenum, New York, 1999.
[21] T. Parasassi, G. De Stasio, A. D'Ubaldo, E. Gratton, Phase fluctuation in phospholipid membranes revealed by laurdan fluorescence, Biophys. J. 57 (1990) 1179–1186.
[22] M.S. Shearman, S.R. Hawtin, V.J. Tailor, The intracellular component of cellular 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) reduction is specifically inhibited by beta-amyloid peptides, J. Neurochem. 65 (1995) 218–227.
[23] M. Suwalsky, Phospholipid bilayers, in: J.C. Salamone (Ed.), Polymeric Materials Encyclopedia, vol. 7, CRC, Boca Raton, FL, 1996, pp. 5073–5078.
[24] M. Masson, B. Spezzatti, J. Chapman, C. Battisti, N. Baumann, Calmodulin antagonists chlorpromazine and W-7 inhibit exogenous cholesterol esterification and sphingomyelinase activity in human skin fibroblast cultures. Similarities between drug-induced and Niemann-Pick type C lipidoses, J. Neurosci. Res. 31 (1992) 84–88.
[25] P. Seeman, The membrane actions of anesthetics and tranquilizers, Pharmacol. Rev. 24 (1972) 583–655.
[26] J.D. Johnson, D.A. Fugman, Calcium and calmodulin antagonists binding to calmodulin and relaxation of coronary segments, J. Pharmacol. Exp. Ther. 226 (1983) 330–334.
[27] T. Ogiso, H. Masuda, S. Oue, Effect of drugs on human erythrocytes. 4. Protecting effect of dextran on drug-induced hemolysis, Biochem. Pharmacol. 27 (1978) 1263–1268.
[28] M.P. Sheetz, S.J. Singer, Biological membranes as bilayer couples. A molecular mechanism of drug-erythrocyte induced interactions, Proc. Natl. Acad. Sci. U. S. A. 71 (1974) 4457–4461.
[29] G. Lim, M. Wortis, R. Mukhopadhyay, Stomatocyte–discocyte–echinocyte sequence of the human red blood cell: evidence for the bilayer-couple hypothesis from membrane mechanics, Proc. Natl. Acad. Sci. U. S. A. 99 (2002) 16766–16769.
[30] S.L. Schrier, A. Zachowski, P.F. Devaux, Mechanisms of amphipath-induced stomatocytosis in human erythrocytes, Blood 79 (1992) 782–786.
[31] W. Nerdal, S.A. Gundersen, V. Thorsen, H. Hoiland, H. Holmsen, Chlorpromazine interactions with glycerophospholipid liposomes studied by magic angle spinning solid state ^{13}C-NMR and differential scanning calorimetry, Biochim. Biophys. Acta 1464 (2000) 165–175.
[32] A.U. Gjerde, H. Holmsen, W. Nerdal, Chlorpromazine interaction with phosphatidylserines: a ^{13}C and ^{31}P solid-state NMR study, Biochim. Biophys. Acta 1682 (2004) 28–37.
[33] A. Enomoto, Y. Takakuwa, S. Manno, A. Tanaka, N. Mohandas, Regulation of erythrocyte ghost membrane mechanical stability by chlorpromazine, Biochim. Biophys. Acta 1512 (2001) 285–290.
[34] G. McEvoy, AHFS drug information, American Society for the Health-System Pharmacists40ºEd., Bethesda, USA, 2006.
[35] S.L. Winski, D.E. Carter, Arsenate toxicity in human erythrocytes: characterization of morphological changes and determination of the mechanism of damage, J. Toxicol. Env. Health, Part A 53 (1998) 345–355.
[36] S. Svetina, D. Kuzman, R.E. Waugh, P. Zibert, B. Zeks, The cooperative role of membrane skeleton and bilayer in the mechanical behaviour of red blood cells, Bioelectrochemistry. 62 (2004) 107–113.