ELSEVIER

Biophysical Chemistry 129 (2007) 36–42

Biophysical Chemistry

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

Effects of lithium on the human erythrocyte membrane and molecular models

Mario Suwalsky [a,*], Paulo Fierro [a], Fernando Villena [b], Carlos P. Sotomayor [c]

[a] *Faculty of Chemical Sciences, University of Concepción, Concepción, Chile*
[b] *Faculty of Biological Sciences, University of Concepción, Chile*
[c] *Institute of Chemistry, Catholic University of Valparaíso, Chile*

Received 1 March 2007; received in revised form 3 May 2007; accepted 7 May 2007
Available online 13 May 2007

Abstract

The mechanism whereby lithium carbonate controls manic episodes and possibly influences affective disorders is not yet known. There is evidence, however, that lithium alters sodium transport and may interfere with ion exchange mechanisms and nerve conduction. For these reasons it was thought of interest to study its perturbing effects upon membrane structures. The effects of lithium carbonate (Li^+) on the human erythrocyte membrane and molecular models have been investigated. The molecular models consisted in bilayers of dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylethanolamine (DMPE), representing classes of phospholipids located in the outer and inner monolayers of the erythrocyte membrane, respectively. This report presents the following evidence that Li^+ interacts with cell membranes: a) X-ray diffraction indicated that Li^+ induced structural perturbation of the polar head group and of the hydrophobic acyl regions of DMPC and DMPE; b) experiments performed on DMPC large unilamellar vesicles (LUV) by fluorescence spectroscopy also showed that Li^+ interacted with the lipid polar groups and hydrophobic acyl chains, and c) in scanning electron microscopy (SEM) studies on intact human erythrocytes the formation of echinocytes was observed, effect that might be due to the insertion of Li^+ in the outer monolayer of the red cell membrane.

Keywords: Lithium; Erythrocyte membrane; Phospholipid bilayer

1. Introduction

Bipolar affective disorder is a serious and enduring mental health problem, which is associated with a high mortality rate worldwide [1]. In spite of a narrow safety margin, lithium salts are widely used in the treatment and prophylaxis [2,3], particularly in the form of Li_2CO_3 [4,5]. The mechanism whereby lithium controls manic episodes and possibly influences affective disorders is not yet known. There is evidence, however, that lithium alters sodium transport and may interfere with ion exchange mechanisms and nerve conduction [6]. Lithium ions are rapidly absorbed from gastrointestinal tract, and plasma lithium peaks are reached 2 to 4 h after administration. Toxicity associated with lithium treatment is highly prevalent as 75 to 90% of patients have signs or symptoms of toxicity during their treatment [7]. The occurrence of toxicity is related to the serum concentration of lithium. Mild toxicity appears at levels up to 2.5 mM, and life-threatening effect is manifest at levels above 3.5 mM [8,9]; however, patients with concentrations as high as 6 mM [10], and even 9.6 mM [11] lithium serum levels following acute intoxication have been reported. The more common side effect involves the central nervous system [4]; chronic lithium treatment affects some signal transduction mechanisms such as cAMP, cGMP, Gi protein, and protein kinase C [3].

Lithium affects ion transport and cell membrane potential by competing with sodium and potassium; these effects may alter neuronal function [12]. Lithium is transported across cell membranes by an exchange diffusion process referred to as Na^+–Li^+ countertransport, so called because sodium, at opposite with the sodium pump, is moved toward the intracellular compartment. This transport mechanism appears to be active in the membranes of cells from many types of tissues and to play a significant role

Abbreviations: SEM, scanning electron microscopy; IUM, isolated unsealed human erythrocyte membrane; LUV, large unilamenar vesicles; *r*, fluorescence anisotropy; GP, fluorescence general polarization; DPH, 1,6-diphenyl-1,3,5-hexatriene; Laurdan, 6-dodecanoyl-2-dimethylaminonaphthalene; DMPC, dimyristoylphosphatidylcholine; DMPE, dimyristoylphosphatidylethanolamine.

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

0301-4622/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bpc.2007.05.003

in the biological disposition of clinically administered lithium [13]. Transmembrane ion movement *via* Na^+–Li^+ countertransport is impeded during lithium treatment because of reduced affinity of the intracellular transport sites for lithium [14]. In fact, lithium is a small ion with virtually no protein binding [8]. Lithium exchange activity has been extensively studied in human red blood cells [14–16]. However, the structural effects of lithium on the human erythrocyte membrane have scarcely been reported.

In the course of *in vitro* systems search for the toxicity screening of chemicals, different cellular models have been applied to examine their adverse effects in isolated organs. This article describes the interaction of lithium carbonate (Li^+) with the human erythrocyte membrane as well as lipids of model membranes. The cell membrane is a diffusion barrier that protects the cell interior; therefore, its structure and functions are susceptible to alterations as a consequence of interactions with foreign species. Erythrocytes were chosen because although less specialized than many other cell membranes, they carry on enough functions in common with them such as active and passive transport, and the production of ionic and electric gradients, to be considered representative of the plasma membrane in general. With the aim to better understand the molecular mechanisms of the interaction of Li^+ with cell membranes we have utilized three well-established models. They regard intact human erythrocytes and molecular models of the erythrocyte membrane. The latter consisted of multibilayers of dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylethanolamine (DMPE), representative of phospholipid classes located in the outer and inner monolayers of the human erythrocyte membrane, respectively [17,18], and large unilamellar vesicles (LUV) of DMPC. The capacity of Li^+ to perturb the multibilayer structures of DMPC and DMPE was determined by X-ray diffraction, while DMPC LUV were studied by fluorescence spectroscopy. Intact human erythrocytes incubated with this compound were observed by scanning electron microscopy. These systems and techniques have been used in our laboratories to determine the interaction with and the membrane-perturbing effects of other metal ions such as Al^{3+} [19], Pb^{2+} [20], Cd^{2+} [21], Au^{3+} [22], Ti^{4+} [23], Fe^{2+}, and Fe^{3+} [24].

2. Materials and methods

2.1. X-ray diffraction studies of phospholipid multilayers

The capacity of Li^+ to perturb the structures of DMPC and DMPE multilayers was evaluated by X-ray diffraction. Synthetic DMPC (lot 140PC-224, MW 677.9), DMPE (lot 140PC-230, MW 635.9) from Avanti Polar Lipids, AL, USA), and Li_2CO_3 (p.a., Merck, Darmstadt, Germany) 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 μl of (a) distilled water and (b) aqueous solutions of Li_2CO_3 in a range of concentrations (0.1 mM to 10 mM). The specimens were X-ray diffracted after 1 h incubation at 37 °C and 60 °C with DMPC and DMPE, respectively, in flat plate cameras. 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 a MBraun PSD-50M linear position-sensitive detector system (Garching, Germany); no correction factors were applied. The experiments were performed at 19±1 °C, which is below the main phase transition temperature of both DMPC and DMPE. Each experiment was repeated three times, and in case of doubts additional experiments were carried out.

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

The influence of Li^+ 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) (Molecular Probe, Eugene, OR, USA) fluorescent probes. 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, as it provides a measure of the 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 physical state of membranes. 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 quantification of the laurdan fluorescence shifts was effected using the general polarization GP concept [25].

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, USA) 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 2 μM, incubating them at 37 °C for 60 min. Fluorescence spectra and anisotropy measurements were performed on a K-2 steady-state and time-resolved spectrofluorometer (ISS Inc., Champaign, IL, USA) interfaced to computer, using the corresponding ISS software for data collection and analysis. Measurements of LUV suspensions were made at 18 °C and 37 °C using 5 mm path-length square quartz cuvettes. Sample temperature was controlled by an external bath circulator (Cole-Parmer, Chicago, IL, USA) and monitored before and after each measurement using an Omega digital thermometer (Omega Engineering Inc., Stanford, CT, USA). Anisotropy measurements were made in the L configuration using Glan Thompson prism polarizers (I.S.S.) in both exciting and emitting beams. The emission was measured using a WG-420 Schott high-pass filter (Schott WG-420, Mainz, Germany) with

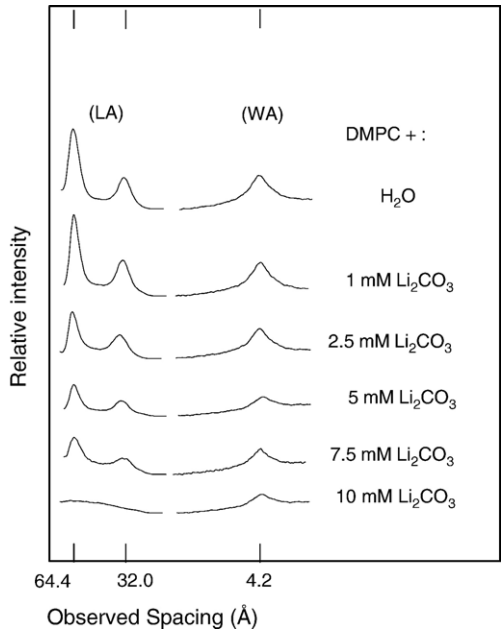

Fig. 1. Microdensitograms from X-ray diffraction patterns of DMPC (dimyristoylphosphatidylcholine) in water and aqueous solutions of Li_2CO_3; (LA) and (WA) correspond to low- and wide-angle reflections, respectively.

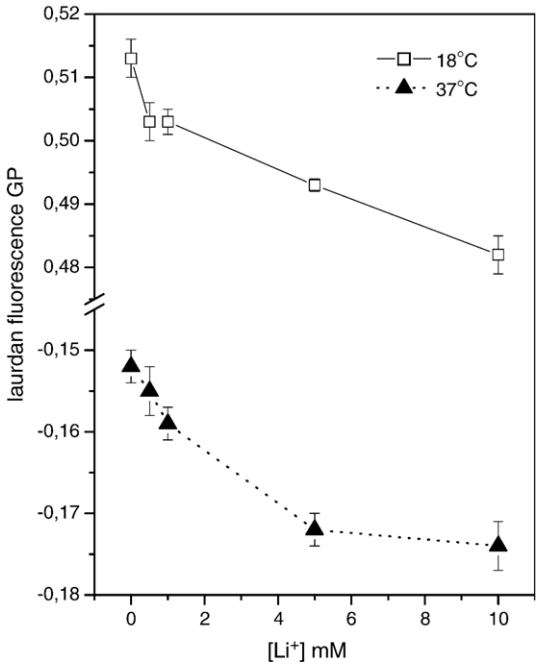

Fig. 3. Effect of Li_2CO_3 on the general polarization (GP) of laurdan embedded in DMPC (dimyristoylphosphatidylcholine) LUV (large unilamellar vesicles) at 18 °C and 37 °C.

negligible fluorescence. DPH fluorescence anisotropy (r) was calculated according to the definition: $r=(I_{\parallel}-I_{\perp})/(I_{\parallel}+2I_{\perp})$ where $I_{\parallel}$ and $I_{\perp}$ are the corresponding vertical and horizontal emission fluorescence intensities with respect to the vertically polarized excitation light [26]. 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 [27]. Li_2CO_3

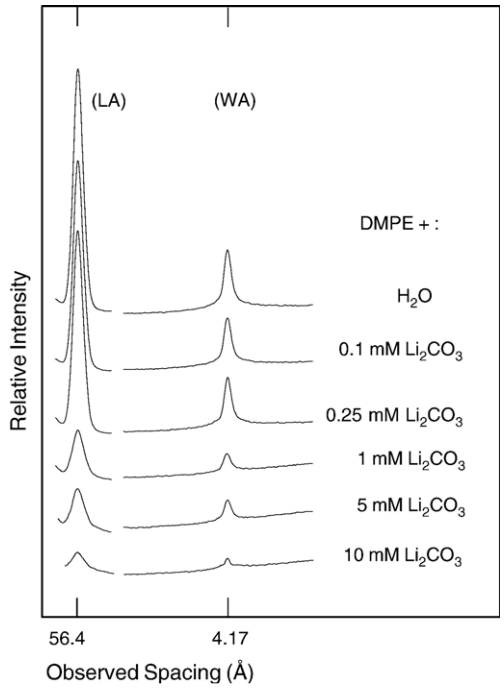

Fig. 2. Microdensitograms from X-ray diffraction patterns of DMPE (dimyristoylphosphatidylethanolamine) in water and aqueous solutions of Li_2CO_3; (LA) and (WA) correspond to low- and wide-angle reflections, respectively.

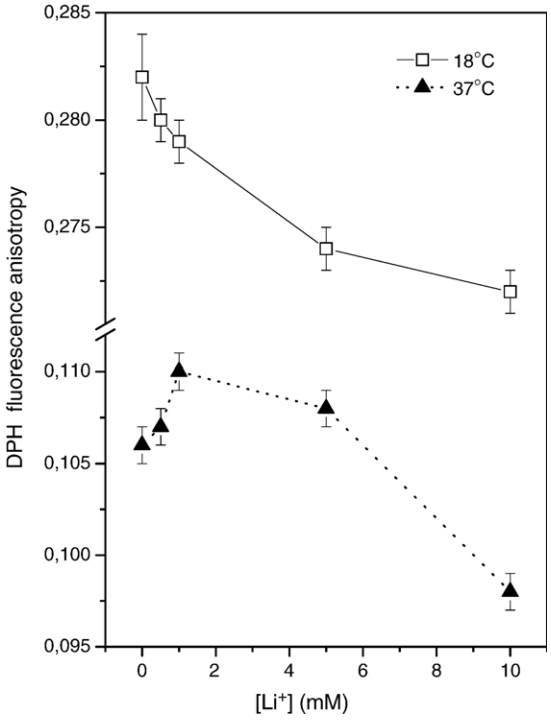

Fig. 4. Effect of Li_2CO_3 on the anisotropy (r) of DPH embedded in DMPC (dimyristoylphosphatidylcholine) LUV (large unilamellar vesicles) at 18 °C and 37 °C.

was incorporated in LUV by addition of adequate (0.1 M) aliquots of Li_2CO_3 solution in order to obtain the different concentrations used in this work. The samples thus prepared were then incubated at 18 °C for *ca.* 15 min. Blank subtraction was performed in all measurements using labeled samples without probes. The data presented in Fig. 2 represents mean values and standard error of ten measurements in two independent samples. Unpaired Student's *t*-test was used for statistical calculations.

2.3. Scanning electron microscopy (SEM) studies on human erythrocytes

In vitro interaction of Li^+ with erythrocytes was attained by incubating red blood cell suspensions derived from healthy human male donors not receiving any pharmacological treatment. Blood samples (0.1 ml) were obtained by puncture of the ear lobule and by aspiration into a tuberculin syringe without a needle containing 50 units/ml heparin in 0.9 ml phosphate buffered saline (PBS), pH 7.4. Red blood cells were then centrifuged for 10 min, washed three times in PBS, resuspended in PBS containing Li^+ in a range of concentrations and incubated for 1 h at 37 °C. Controls were cells resuspended in PBS without Li_2CO_3. Specimens were then fixed overnight at 5 °C by adding one drop of each sample to plastic tubes containing 1 ml of 2.5% glutaraldehyde, washed twice in distilled water, placed on siliconized Al stubs and air dried at 37 °C for 30 min and gold coated for 3 min at 13.3 Pascal in a sputter device (Edwards S 150, Sussex, England). Specimens were examined in a JEOL (JEM 6380 LB, Japan) SEM. The

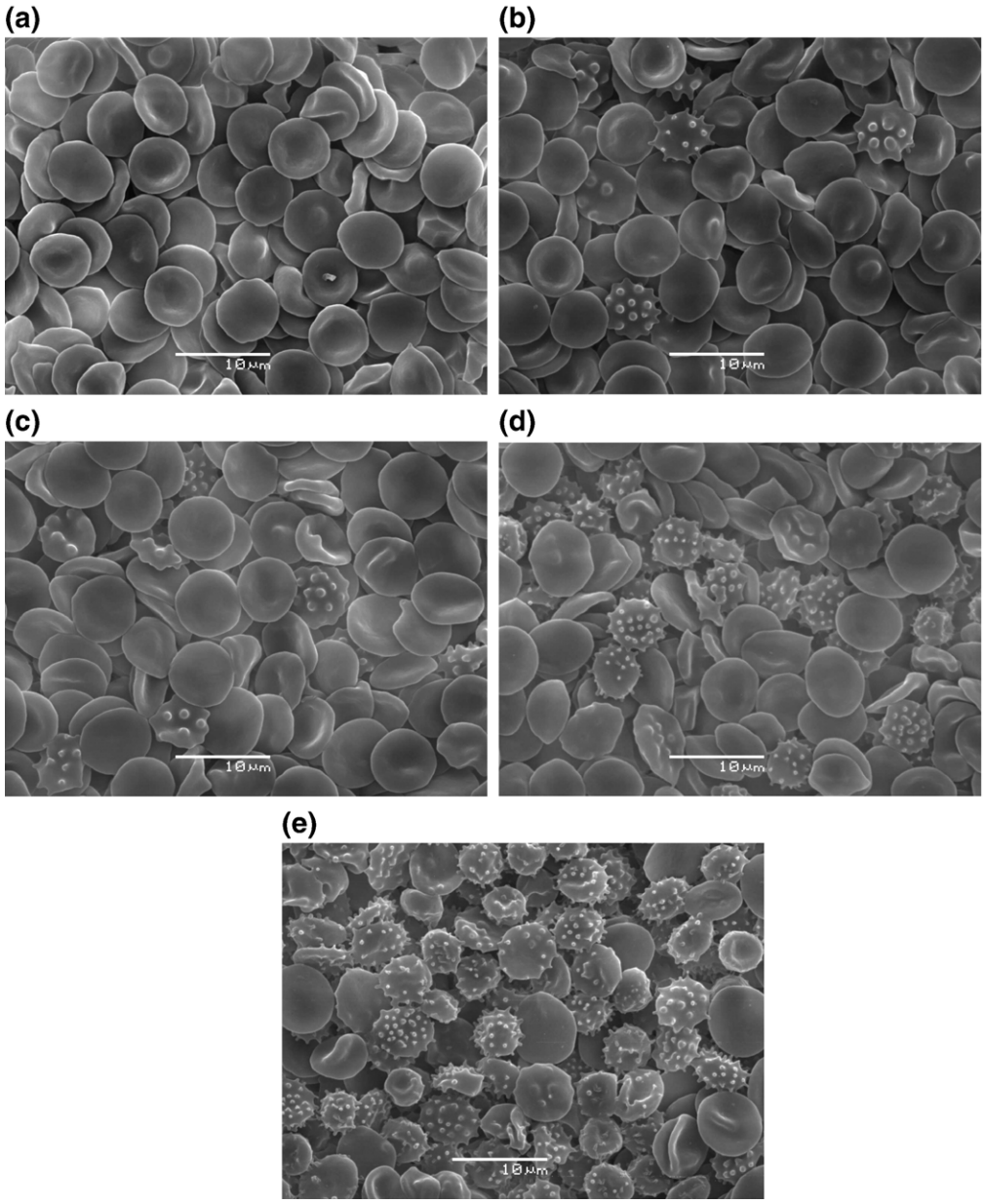

Fig. 5. Effects of Li_2CO_3 on the morphology of human erythrocytes. Shown are scanning electron microscope images of: (a) untreated erythrocytes, erythrocytes incubated with (b) 0.25 mM, (c) 1 mM, (d) 5 mM, and (e) 10 mM Li_2CO_3.

data was expressed as mean±standard deviation of 50 cell counts.

3. Results

3.1. X-ray diffraction studies of phospholipid multilayers

Fig. 1 exhibits the results obtained by incubating DMPC with water and Li_2CO_3. As expected, water altered the structure of DMPC, as its bilayer width increased from about 55 Å in its dry crystalline form [28] to 64 Å when immersed in water, and its low-angle reflections, 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 which corresponds to the average distance between fully extended acyl chains organized with rotational disorder in hexagonal packing. These results were indicative of the fluid state reached by DMPC bilayers. Fig. 1 discloses that after exposure to 2.5 mM and higher concentrations of Li_2CO_3 there was a gradual weakening of the low- and wide-angle DMPC reflection intensities (indicated as LA and WA in the figure, respectively) which at 10 mM practically disappeared the low-angle reflections while that of 4.2 Å remained although with a considerable reduced intensity. From these results it can be concluded that Li^+ produced a significant structural perturbation of DMPC bilayers, particularly to the lipid polar group region. Results from similar experiments with DMPE are presented in Fig. 2. As reported elsewhere, water did not significantly affect the bilayer structure of DMPE [28]. It can be observed that increasing concentrations of Li^+ gradually reduced DMPE reflection intensities; however all of them were still present at the highest 10 mM Li_2CO_3 concentration assayed.

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

Li^+ concentration-dependent effects on DMPC LUV were explored at two different depths of the lipid bilayer: at the hydrophilic/hydrophobic interface level, estimated from the laurdan fluorescence spectral shift through the general polarization (GP) parameter, and in the deep hydrophobic core, determined by the DPH steady-state fluorescence anisotropy (r). Fig. 3 shows that Li^+ induced a decrease of the general polarization at 18 °C and 37 °C, implying an increase in the environmental molecular dynamics and/or water penetration at the glycerol backbone level, a result that can be attributed to a disordering effect induced by Li^+ on DMPC polar head groups. On the other hand, the effect of Li^+ on the DPH anisotropy shows a different pattern at both temperatures. As it can be seen in Fig. 4, increasing concentrations of Li^+ cause a decrease of the fluorescence anisotropy at 18 °C, when the bilayer is in the gel–crystalline state, while a biphasic pattern is observed at 37 °C, when the bilayer is in the much more fluid liquid–crystalline state: initial increase of this parameter occurred up to 0.1 mM Li_2CO_3, followed by a decrease at higher concentrations. These latter results imply that Li^+ first ordered the phospholipid acyl chain packing arrangement and afterwards disordered them as the Li^+ concentration increased.

3.3. Scanning electron microscopy (SEM) studies on human erythrocytes

The SEM examinations of red blood cells incubated with Li_2CO_3 indicated that they presented cell shape abnormalities of the echynocitosis type, consisting in the formation of blebs or protuberances on the erythrocyte surface. As it can be observed in Fig. 5, the cell shape change increased with Li^+ concentration. In fact, 0.25 mM Li_2CO_3 induced a 10%, 1 mM 20%, 5 mM 45–50% and 10 mM 70% echynocitosis.

4. Discussion

In order to understand the structural effects of Li^+ on the human erythrocyte membrane Li_2CO_3 was incubated with intact erythrocytes and bilayers built-up of DMPC and DMPE, phospholipid classes present in the outer and inner monolayers of the erythrocyte membrane, respectively. Analysis by X-ray diffraction showed that 2.5 mM and higher concentrations of Li_2CO_3 induced structural perturbations of the polar head groups and to a lower extent of the hydrophobic acyl regions of DMPC. On the other hand, the effects on DMPE bilayers were almost the same, although the perturbing effects were observed at higher Li_2CO_3 concentrations. Chemically the two lipids only differ 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: their acyl chains are mostly parallel and extended with the polar groups lying perpendicularly to them; however, DMPE molecules pack tighter than those of DMPC. This effect, due to the DMPE smaller polar group and higher effective charge, makes for a very stable multilayer arrangement that is not significantly perturbed by the presence of water [28]. On the other hand, the gradual hydration of DMPC bilayers leads to water filling the highly polar interbilayer spaces. Consequently, there is an increase in its bilayer repeat from 54.5 Å when dry to 64.5 Å when fully hydrated at a temperature below that of its main transition. This condition promoted the incorporation of Li^+ ions into DMPC highly polar interbilayer space and the ensuing molecular perturbation of the phospholipid bilayer structure. This hypothesis is supported by the fluorescence spectroscopy measurements on DMPC LUV. In fact, at about the same concentration range and temperature (18 °C) as the X-ray experiments, as well as at 37 °C, Li^+ induced structural perturbations in both the polar and acyl chain regions of DMPC bilayers.

The Li^+ induced mild decrease in Laurdan fluorescence parameter GP indicates a small, although significant, increase in the membrane hydration at the hydrophilic hydrophobic interface, *i.e.* the phospholipid glycerol backbone level, where laurdan fluorescent moiety is located. The effect of a monovalent cation on membrane surface properties has been hypothesized by Kraayenhof et al. [29] to be related to its own hydration tendency and potency to stabilize or destabilize the hydrogen-bond network along membrane surface, *i.e.* the

ordered water interfacial hydration layer, suggesting that a small hydrated ion can fit well within the water lattice without disrupting the hydrogen-bond network. The above mentioned Li^+ effect, sensed by laurdan, can be rationalized in this context as a small perturbation in the ordered water interfacial hydration layer which influences the hydration and/or molecular dynamics at the neighbor hydrophilic hydrophobic interface. The latter interface is assumed to be at a somewhat deeper level of the interfacial layer. This perturbation also influences, although to a lesser extent, the fatty acid acyl chain packing order, as sensed by the milder change in DPH fluorescence anisotropy. However, our previous results [20,21,23], and of other authors [30] indicate a lack of correlation of head group hydration with acyl chain order in different membrane models.

According to the bilayer couple hypothesis [31], shape changes are induced in red cells due to the insertion of foreign species in either the outer or the inner monolayer of the erythrocyte membrane. Thus, spiculated shapes (echinocytes) are observed in the first case while cup shapes (stomatocytes) are produced in the second due to the differential expansion of the corresponding monolayer. Given the extent of the interaction of Li^+ with DMPC, a class of lipid preferentially located in the outer monolayer of the erythrocyte membrane, echinocytes were expected of erythrocytes incubated with Li_2CO_3. SEM examination of specimens showed that in fact Li^+ induced a change of shape of the normal biconcave erythrocytes. Although some echinocytes were observed with 0.1 mM Li_2CO_3, the number of equinocytes considerably increased with higher Li^+ concentration, reaching a 50% of the cells with 5 mM Li_2CO_3, and 70% with 10 mM Li_2CO_3.

Studies on the interaction of lithium with the human erythrocyte membrane are indeed very scanty. SEM observations indicated that Li^+ induced shape alterations with tendency to crenation, an effect that was not observed with Na^+ or K^+ [32]. These authors disregarded the interaction of Li^+ with phosphatidylcholines and phosphatidylethanolamines present in the erythrocyte membrane. In another study, carried out by NMR, Pettegrew et al. [33] reported that lithium did not interact significantly with hemoglobin, the erythrocyte membrane, or artificial phospholipids membranes. However, using fluorescence anisotropy Pettegrew et al. [34] concluded that 2 mM Li^+ significantly altered molecular dynamics on the erythrocyte membrane surface, with less striking changes in the hydrocarbon core. However, the interpretation of their findings was based on that hydrated Li^+ altered the electrostatic interaction of membrane surface molecules, as well as the surrounding water structure. Additional studies have been performed on phospholipid bilayers, particularly with phosphatidylcholine and phosphatidylserine artificial membranes [35–39]. Data indicated that Li^+ ions interact with choline and serine groups [36], binding to the phosphate and carboxylate groups of saturated and unsaturated phosphatidylserines loosing their water of hydration [38,39]. Li^+ chemistry can be largely understood on the basis of its exceptionally small radius (0.6 Å) having thus a large effective charge. In this context, we postulate that electrostatic interactions of Li^+ ions with choline–polar groups located in the outer monolayer of the erythrocyte membrane are at least one of the major factors responsible for the cell shape change into echinocytes. This conclusion is also being supported by the studies performed by NMR and fluorescence measurements on human erythrocytes [40]. Such Li^+-induced effects may result in perturbations of receptor function at the membrane surface, and modulation of ion channel activity at the membrane interior. These findings may provide an insight into the possible molecular mechanism for the therapeutic action of Li^+.

Acknowledgements

This work was supported by grants from FONDECYT (1060990) and UDEC-PUCV (205.024.031-1S).

References

[1] M. Shaw, The role of lithium clinics in the treatment of bipolar disorder, Nurs. Times 100 (2004) 42–46.
[2] S.M. Carney, G.M. Goodwin, Lithium — a continuing story in the treatment of bipolar disorder, Acta Psychiatr. Scand. Suppl. 426 (2005) 7–12.
[3] L. Carbonell, M.L. Cuffi, J. Forn, Effect of chronic lithium treatment on the turnover of alpha2-adrenoceptors after chemical inactivation in rats, Eur. Neuropsychopharmacol. 14 (2004) 497–502.
[4] Y. Suraya, K.Y. Yoong, Lithium neurotoxicity, Med. J. Malasya 56 (2001) 378–381.
[5] J. Emami, N. Tavakoli, A. Movahedian, Formulation of sustained — release lithium carbonate matriz tablets: influence of hydrophilic materials no the release rate and in vitro–in vivo evaluation, J. Pharm. Sci. 15 (2004) 338–344.
[6] P.W. Long, Lithium Carbonate, 2005, pp. 1–9, www.mentalhealth.com.
[7] A.L. Webb, D.A. Solomon, C.E. Ryan, Lithium levels and toxicity among hospitalized patients, Psychiatr. Serv. 52 (2001) 229–231.
[8] R. Peces, A. Pobes, Effectiveness of haemodialysis with high-flux membranes in the extracorporeal therapy of life-threatening acute lithium intoxication, Nephrol. Dial. Transplant. 16 (2001) 1301–1303.
[9] J. Borras Blasco, A. Murcia Lopez, I. Romero Crespo, A. Sirvent Pedreno, A. Navarro Ruiz, Acute intoxication with sustained-release lithium carbonate tablets. A propos of a case, Farm. Hosp. 29 (2005) 140–143.
[10] R.E. Dupuis, A.A. Cooper, L.J. Rosamond, S. Campbell-Bright, Multiple delayed peak lithium concentrations following acute intoxication with an extended-release product, Ann. Pharmacother. 30 (1996) 356–360.
[11] A. Jaeger, P. Sauder, J. Kopferschmitt, L. Tritschh, F. Flesch, When should dialysis be performed in lithium poisoning? A kinetic study in 14 cases of lithium poisoning, Clin. Toxicol. 31 (1993) 429–447.
[12] J. Gill, H. Singh, K. Nugent, Acute lithium intoxication and neuroleptic malignant syndrome, Pharmacotherapy 23 (2003) 811–815.
[13] A.G. Mallinger, E. Frank, M.E. Thase, C.S. Dippold, D.J. Kupfer, Low rate of membrane lithium transport during treatment correlates with outcome of maintenance pharmacotherapy in bipolar disorder, Neuropsychopharmacology 16 (1997) 325–332.
[14] A.G. Mallinger, J.M. Himmelhoch, M.E. Thase, C. Dippold, S. Knopf, Reduced cell membrane affinity for lithium ion during maintenance treatment of bipolar disorder, Biol. Psychiatry 27 (1990) 795–798.
[15] M. Cirillo, M. Laurenzi, W. Panarelli, M. Trevisan, J. Stamler, Prospective analysis of traits related to 6-year change in sodium–lithium countertransport, Hypertension 33 (1999) 887–893.
[16] D. Mohanty, Red blood cell membrane — is it a mirror for systemic non-hematological genetic disorders? Indian J. Hum. Genet. 10 (2004) 39–40.
[17] J.M. Boon, B.D. Smith, Chemical control of phospholipids distribution across bilayer membranes, Med. Res. Rev. 22 (2002) 251–281.
[18] P.F. Devaux, A. Zachowsky, Maintenance and consequences of membrane phospholipids asymmetry, Chem. Phys. Lipids 73 (1994) 107–120.
[19] M. Suwalsky, B. Norris, T. Kiss, P. Zatta, Effects of Al(III) speciation on cell membranes and molecular models, Coord. Chem. Rev. 228 (2002) 285–295.

[20] M. Suwalsky, F. Villena, B. Norris, F. Cuevas, C.P. Sotomayor, P. Zatta, Effects of lead on the human erythrocyte membrane and molecular models, J. Inorg. Biochem. 97 (2003) 308–313.
[21] M. Suwalsky, F. Villena, B. Norris, F. Cuevas, C.P. Sotomayor, Cadmium-induced changes in the membrane of human erythrocytes and molecular models, J. Inorg. Biochem. 98 (2004) 1061–1066.
[22] M. Suwalsky, P. Zambenedetti, E. Carpene, M. Ibnlkayat, W. Wittkowski, P. Zatta, Effects of chronic gold treatment in the mouse brain and in membrane models, J. Inorg. Biochem. 98 (2004) 2080–2086.
[23] M. Suwalsky, F. Villena, B. Norris, M.A. Soto, C.P. Sotomayor, L. Messori, P. Zatta, Structural effects of titanium citrate on the human erythrocyte membrane, J. Inorg. Biochem. 99 (2005) 764–770.
[24] M. Suwalsky, F. Martínez, J. Grzyb, K. Strzalka, Iron affects the structure of cell membrane models, Chem. Phys. Lipids 134 (2005) 69–77.
[25] T. Parasassi, E. Gratton, Membrane lipid domains and dynamics as detected by laurdan fluorescence, J. Fluoresc. 5 (1995) 59–69.
[26] J.R. Lakowicz, Principles of Fluorescence Spectroscopy, Plenum, New York, 1999.
[27] 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.
[28] M. Suwalsky, in: J.C. Salamone (Ed.), Polymeric materials encyclopedia, Phospholipid Bilayers, vol. 7, CRC, Boca Raton, FL, 1996, pp. 5073–5078.
[29] R. Kraayenhof, G.J. Sterk, H.W. Sang, K. Krab, R.M. Epand, Monovalent cations differentially affect membrane surface properties and membrane curvature, as revealed by fluorescent probes and dynamic light scattering, Biochim. Biophys. Acta 1282 (1996) 293–302.
[30] C. Ho, S.J. Slater, C.D. Stubbs, Hydration and order in lipid bilayers, Biochemistry 34 (1995) 6188–6195.
[31] M.P. Sheetz, S.J. Singer, Biological membranes as bilayer couples. A molecular mechanism of drug-induced interactions, Proc. Natl. Acad. Sci. U. S. A. 75 (1974) 4457–4461.
[32] B. Zimmermann, D.M. Soumpasis, Effects of monovalent cations on red cell shape and size, Cell Biophys. 7 (1985) 115–127.
[33] J.W. Pettegrew, J.F.M. Post, K. Panchalingam, G. Withers, D.E. Woessner, ^{7}Li NMR study of normal human erythrocytes, J. Magn. Reson. 71 (1987) 504–519.
[34] J.W. Pettegrew, J.W. Short, R.D. Woessner, S. Strychor, D.W. McKeag, J. Armstrong, N.J. Minshew, A.J. Rush, The effect of lithium on the membrane molecular dynamics of normal human erythrocytes, Biol. Psychiatry 22 (1987) 857–871.
[35] E.T. Fossel, M.M. Sarasua, K.A. Koehler, A lithium-7 NMR investigation of the lithium ion interaction with phosphatidylcholine–phosphatidylglycerol membranes. Observations of calcium and magnesium ion competition, J. Magn. Reson. 64 (1985) 536–540.
[36] M. Roux, M. Bloom, Ca^{2+}, Mg^{2+}, Li^{+}, Na^{+}, and K^{+} distributions in the headgroup region of binary membranes of phosphatidylcholine and phosphatidylserine as seen by deuterium NMR, Biochemistry 29 (1990) 7077–7089.
[37] H.L. Casal, H.H. Mantsch, F. Paltauf, H. Hauser, Infrared and ^{31}P-NMR studies of the effect of Li^{+} and Ca^{2+} on phosphatidylserines, Biochim. Biophys. Acta 919 (1987) 275–286.
[38] H.L. Casal, H.H. Mantsch, H. Hauser, Infrared studies of fully hydrated saturated phosphatidylserine bilayers. Effect of Li^{+} and Ca^{2+}, Biochemistry 26 (1987) 4408–4416.
[39] H.L. Casal, A. Martin, H.H. Mantsch, F. Paltauf, H. Hauser, Infrared studies of fully hydrated unsaturated phosphatidylserine bilayers. Effect of Li^{+} and Ca^{2+}, Biochemistry 26 (1987) 7395–7401.
[40] D. Mota de Freitas, L. Amari, Ch. Srinivasan, Q. Rong, R. Ramasamy, A. Abraha, C.F.G.C. Geraldes, M. Boyd, ^{7}Li NMR study of normal human erythrocytes, Biochemistry 33 (1994) 4101–4110.