The Anticancer Drug Chlorambucil Interacts with the Human Erythrocyte Membrane and Model Phospholipid Bilayers

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Chlorambucil, Anticancer Drug, Erythrocyte Membrane, Phospholipid Bilayer

The plasma membrane has gained increasing attention as a possible target of antitumor drugs. It has been reported that they act as growth factor antagonists, growth factor receptor blockers, interfere with mitogenic signal transduction or exert direct cytotoxic effects. Chlorambucil (4-[*p*-(bis[2-chloroethyl]amino)phenyl]butyric acid) is an alkylating agent widely used in the treatment of chronic lymphocytic leukaemia. Contradictory reports have been published concerning its interaction with cell membranes. Whereas a decrease in the fluidity of Ehrlich ascite tumor cells has been adduced, no evidences were found that chlorambucil changes membrane lipid fluidity and alkylating agents had effects in these systems even at highly toxic concentrations. Our results showed that chlorambucil at a dose equivalent to its therapeutical concentration in the plasma (3.6 μM) caused the human erythrocyte membrane to develop cup-shaped forms (stomatocytes). Accordingly to the bilayer couple hypothesis, this means that the drug is inserted into the inner monolayer of the erythrocyte membrane, a conclusion supported by X-ray diffraction performed on multilayers of dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylethanolamine (DMPE), representative of phospholipid classes located in the outer and inner monolayers of the erythrocyte membrane, respectively. It is concluded that the cytotoxic effect of chlorambucil might be due to alteration of the structure and therefore of the physiological properties of cell membranes such as fluidity, permeability, receptor and channel functions.

Introduction

Alkylating agents have been used for more than 30 years as cytotoxic drugs to treat many malignancies (Shulman, 1993). These compounds replace the hydrogen of a reacting chemical by an alkyl group. It is generally believed that DNA alkylation is responsible for the antitumor effect of alkylating drugs (Povirk and Shuker, 1994). Although there is good correlation between DNA cross-linking and growth inhibition, clear-cut evidence that this is the only essential event leading to blockade of cell proliferation by bifunctional alkylating agents is still lacking (Grunicke *et al.*, 1985a). The plasma membrane as a possible target of antitumor drugs has gained increasing attention (Luxo *et al.*, 1996; Speelmans *et al.*, 1996; Marutaka *et al.*, 1994), where they act as growth factor antagonists, growth factor receptor blockers, interfere with mitogenic signal transduction or exert direct cytotoxic effects (Grunicke and Hoffmann, 1992). Furthermore, drugs which are considered to exert their antiproliferative effect by interacting with DNA cause structural and functional membrane alterations which may be essential for growth inhibition by these agents (Grunicke *et al.*, 1985b). However, the detailed mechanism by which alkylating drugs inhibit cell division is still unclear. Chlorambucil (4-[*p*-(bis[2-chloroethyl]-amino)phenyl]butyric acid, or leukeran), is a bifunctional nitrogen mustard (Fig. 1). It is an alkylating agent used in the treatment of chronic lymphocytic leukaemia, the most prevalent form of this disease in Western countries (Löf *et al.*,

Abbreviations: DMPC, dimyristoylphosphatidylcholine; DMPE, dimyristoylphosphatidylethanolamine; LUV, large unilamellar vesicles; DPH, 1,6-diphenyl-1,3,5-hexatriene; Laurdan, 6-dodecanoyl-2-dimethylaminonaphthalene; r, fluorescence anisotropy; GP, general polarization; SEM, scanning electron microscopy.

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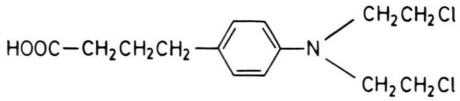

Fig. 1. Structural formula of chlorambucil.

1997). Fluorescence spectroscopy has shown that it decreased the fluidity of Ehrlich ascite tumor cell membranes, an effect due to direct interaction with the plasma membrane, expressed at all concentrations of the drug which inhibited cell proliferation (Grunicke *et al.*, 1983). Additional studies yielded no evidence for a change in membrane lipid fluidity, and it was concluded that bifunctional alkylating agents caused the observed effects by forming membrane protein cross-links (Grunicke *et al.*, 1985b). On the other hand, spin-label methods showed that alkylating agents had no effect on the fluidity of phospholipid membranes or of Ehrlich ascites tumour cells membranes even at highly toxic concentrations (Ankel *et al.*, 1986). The controversy surrounding the interaction of chlorambucil with cell membranes encouraged us to undertake a study concerning this subject. This paper describes the interaction of chlorambucil with the human erythrocyte membrane and models constituted by phospholipid multilayers and large unilamellar vesicles (LUV). These systems have been used in our laboratories to determine the interaction and perturbing effects on membranes by other anticancer drugs such as tamoxifen (Suwalsky *et al.*, 1998) and adriamycin (Suwalsky *et al.*, 1999). The interactions of chlorambucil with human erythrocytes were examined by scanning electron microscopy (SEM) to detect shape changes induced by the drug. The multilayers consisted of the phospholipids dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylethanolamine (DMPE), representative of phospholipid classes located in the outer and inner monolayers of the human erythrocyte membrane, respectively (Devaux and Zachowsky, 1994). Given the amphipathic character of chlorambucil and of both phospholipids their interactions were assayed in hydrophobic and aqueous media in a range of concentrations. Chlorambucil perturbation of the DMPC and DMPE multilayer structures was determined by X-ray diffraction (Suwalsky, 1988). The effect of the drug on the physical properties of DMPC LUV was studied evaluating DPH steady state fluorescence anisotropy and laurdan fluorescence spectral shifts.

Materials and Methods

Scanning electron microscope (SEM) studies on human erythrocytes

Blood samples taken from clinically healthy male adult donors by puncture of the ear lobe disinfected with 70% ethanol were incubated with chlorambucil. Two drops of blood were collected in a plastic tube containing 1 ml of saline (0.9% NaCl) at 5 °C. This solution was used to prepare the following samples: a) control, by mixing 0.1 ml with 0.9 ml of saline, and b) 3.6 μM chlorambucil by mixing 0.1 ml with 0.9 ml of chlorambucil in adequate concentration. This concentration is that present in plasma when the drug is administered to patients (American Hospital Formulary Service Drug Information 95, 1995).The samples were incubated at 37 °C for 1 h and then fixed with glutaraldehyde adding one drop of each sample to a tube containing 1 ml of 2.5% glutaraldehyde in saline, reaching a final fixation concentration of about 2.4%. After resting overnight at 5 °C the fixed samples were directly placed on Al stubs, air dried at 37 °C for half to one h and gold coated for 3 min at 10^{-1} Torr in a sputter device (Edwards S150, Sussex, England). Resulting specimens were examined in an Etec Autoscan SEM (Etec Corp., Hayward, CA, USA).

X-ray diffraction analysis of phospholipid multilayers

Synthetic DMPC (lot 80H-8371 A grade MW 677.9), DMPE (lot 13H-83681 A grade MW 635.9) and chlorambucil (lot 44H2605, MW 304.2) from Sigma (St. Louis, MO, USA) were used without further purification. About 5 mg of each phospholipid were mixed with the corresponding weight of chlorambucil in order to attain DMPC:chlorambucil and DMPE:chlorambucil powder mixtures in the molar ratios of 10:1, 5:1 and 1:1. Each mixture was dissolved in chloroform:methanol 3:1 v/v and left to dry. The recrystallized samples were placed in special glass capillaries 0.7 mm diameter (Glas Technik & Konstruktion, Berlin, Germany). They were diffracted in Debye-Scherrer cameras of 114.6 mm diameter (Philips, The Netherlands) and

flat-plate cameras built by us with 0.25 mm diameter glass collimators provided with rotating devices. The same procedure was followed with samples of each phospholipid and chlorambucil. The aqueous specimens were prepared in 1 mm diameter glass capillaries mixing each phospholipid and chlorambucil in the proportions described above. Each capillary was then filled with about 100 μl of distilled water. These specimens were X-ray diffracted 2 days after preparation 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 Philips PW1140 X-ray generator (The Netherlands) was used. The relative reflection intensities on films were measured by peak-integration in Joyce-Loebl MKIIICS (England) and Bio Rad GS-700 (Hercules, CA, USA) microdensitometers using the Bio-Rad Molecular Analyst/PC image software. The experiments in water were performed at 17 ± 2 °C, which is below the main transition temperature of both DMPC and DMPE. Higher temperatures would have induced transitions to more fluid phases making it harder to detect the structural changes produced by chlorambucil.

Fluorescence measurements on large unilamellar vesicles (LUV)

DMPC LUV suspended in water were prepared by extrusion of frozen and thawed multilamellar liposome suspension (final lipid concentration 0.5 mM) through two stacked polycarbonate filters of 400 nm pore size (Nucleopore, Corning Costar Corp., Cambridge, MA, USA) under nitrogen pressure at 10 °C above the lipid transition temperature. DPH and laurdan were incorporated into LUV by addition of small aliquots of concentrated solutions of the probe in tetrahydrofurane and ethanol respectively to LUV suspensions and gently shaken for about 30 min. Fluorescence spectra and anisotropy measurements were respectively performed in a Spex Fluorolog (Spex Industries Inc., Edison, NJ, USA) and in a phase shift and modulation Greg-200 steady-state and time-resolved spectrofluorometer (I. S. S. Inc., Champaign, IL, USA), both interfaced to computers. Software from I. S. S. was used for data collection and analysis. Measurements of LUV suspensions were made at 18 °C in 10 mm path-length square quartz cuvettes. Sample temperature was controlled by an external bath circulator (Cole Parmer, Chicago, IL, USA) and measured prior and after each measurement using a digital thermometer (Omega Engineering. Inc., Stanford, CT, USA). Anisotropy measurements were made in the "L" configuration using prism polarizers (Glan Thompson, I. S. S.) in both exciting and emitting beams. The emission was measured across a high pass filter (Schott WG-420, Mainz, Germany) with negligible fluorescence. Laurdan fluorescence spectral shifts were quantified through the General Polarization (GP) concept which was evaluated by GP=(Ib-Ir)/(Ib+Ir), where Ib and Ir are the 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 (Parasassi and Gratton, 1995). Chlorambucil was incorporated in LUV suspensions by addition of small aliquots of a concentrated ethanol solution and incubated at 40 °C for ca. 15 min. Samples with probes, including ethanol, but without chlorambucil showed no variation in the measured parameters during periods longer than those employed in the experiments. Blank subtraction was performed in all measurements using unlabelled samples without probes.

Results

Scanning electron microscopy (SEM) studies on human erythrocytes

SEM of red cells incubated with 3.6 μM revealed abnormalities in their shapes. In contrast to the normal discoid erythrocyte profile (Fig. 2a) a great number of chlorambucil treated cells underwent stomatocytic shape changes, i.e., evagination of one surface and invagination of the opposite face (Fig. 2b). The assayed concentration of chlorambucil is equivalent to that found in plasma after its therapeutical administration.

X-ray diffraction analysis of phospholipid multilayers

The molecular interactions of chlorambucil with multilayers of the phospholipids DMPC and

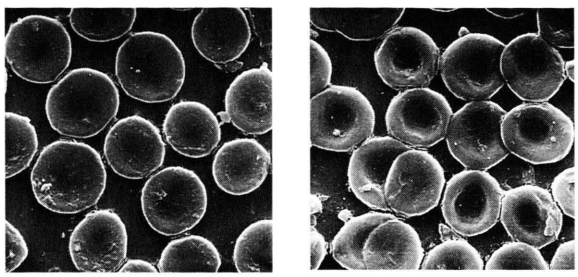

Fig. 2. Scanning electron microscope (SEM) images of human erythrocytes, 3000x; (a) Control; (b) incubated with 3.6 μM chlorambucil.

DMPE were investigated in hydrophobic and aqueous media. Fig. 3A shows a comparison of the diffraction patterns of DMPC and chlorambucil and of their 10:1, 5:1 and 1:1 molar mixtures after interacting and recrystallizing from $CHCl_3$: CH_3OH 3:1 (v/v). Results indicated that the X-ray pattern of DMPC was affected by the drug: in the 10:1 molar ratio, chlorambucil perturbed the high angle region of DMPC to a large extent and its intense reflections of 4.30 Å and 4.19 Å were replaced by a strong band of 4.2 Å. Increasing the drug concentration decreased DMPC reflection intensities, although the phospholipid bilayer width remained practically constant at about 55 Å. The main reflections of chlorambucil were present in the 1:1 and 5:1 molar mixtures. These results implied that only a limited amount of chlorambucil penetrated the phospholipid bilayer perturbing mainly its acyl chain packing arrangement. Fig. 3B shows the results obtained after DMPC, chlorambucil and their molar mixtures in the same ratios as above were immersed in distilled water. DMPC bilayer width increased from 55 Å when dry to 64.0 Å. Its reflections were reduced to only the first three orders of the bilayer width and a relatively intense reflection of 4.2 Å. The latter arose from the stiff and fully extended acyl chains organized with rotational disorder in an hexagonal lattice. Increasing proportions of chlorambucil in the mixtures produced the following effects: a) a gradual decrease of DMPC reflection intensities in both the low and high angle regions, which were of the order of 64% at the highest concentrations of the drug; b) an expansion of the bilayer width up to 70.4 Å, attained in the 5:1 molar ratio, which decreased to 66.6 Å in the 1:1 molar mixture, and c) the presence of the drug reflections in the 1:1 and 5:1 samples. These results indicated that chlorambucil, despite its low solubility in water, interacted with DMPC perturbing its bilayer structure.

Results of the interaction of chlorambucil with DMPE in the hydrophobic medium are shown in Fig. 3C. As reported elsewhere (Suwalsky *et al.*, 1991), DMPE presents two polymorphic forms. One form (Lc_1) has the acyl chains extended and parallel to the bilayer normal, and its bilayer width is about 52 Å. In the other form (Lc_2) the acyl chains are tilted by about 30º and the bilayer width is nearly 44 Å. Chlorambucil induced the following effects in DMPE: a) an Lc_1 to Lc_2 phase transition; and b) a decrease of its reflection intensities. However, the bilayer width of the DMPE Lc_2 form remained practically constant at 45.0 Å. On the other hand, reflections from the drug were observed only in the 1:1 molar mixture. Fig. 3D illustrates the interaction of chlorambucil with DMPE in water; the drug did not evoke a phase transition in DMPE, which remained throughout in the Lc_1 form. However, it induced a gradual reduction of the lipid reflection intensities as its molar ratio increased, reaching an average of an 82% reduction in the high angle reflection intensities. The strongest reflections of chlorambucil were observed only in its 1:1 molar mixture with DMPE. These results implied an extensive interaction of the drug with DMPE, somewhat higher than that observed with DMPC. This was rather surprising in view of the fact that DMPE molecules pack closer and tighter than those of DMPC, its structure being more difficult to penetrate and disturb (Suwalsky, 1988).

Fluorescence measurements on large unilamellar vesicles (LUV)

The structural effects of chlorambucil on DMPC LUV were determined at the acyl chain deep hydrophobic and hydrophilic/hydrophobic interface regions of the phospholipid bilayer, by evaluation of DPH steady state fluorescence anisotropy (r) and laurdan general polarization (GP). As shown in Table I, increasing concentrations of chlorambucil up to 1 mM decreased r and GP values to the order of 16% and 30%, respectively. The DPH steady state anisotropy is primarily related to the rotational motion restriction due to the hydrocar-

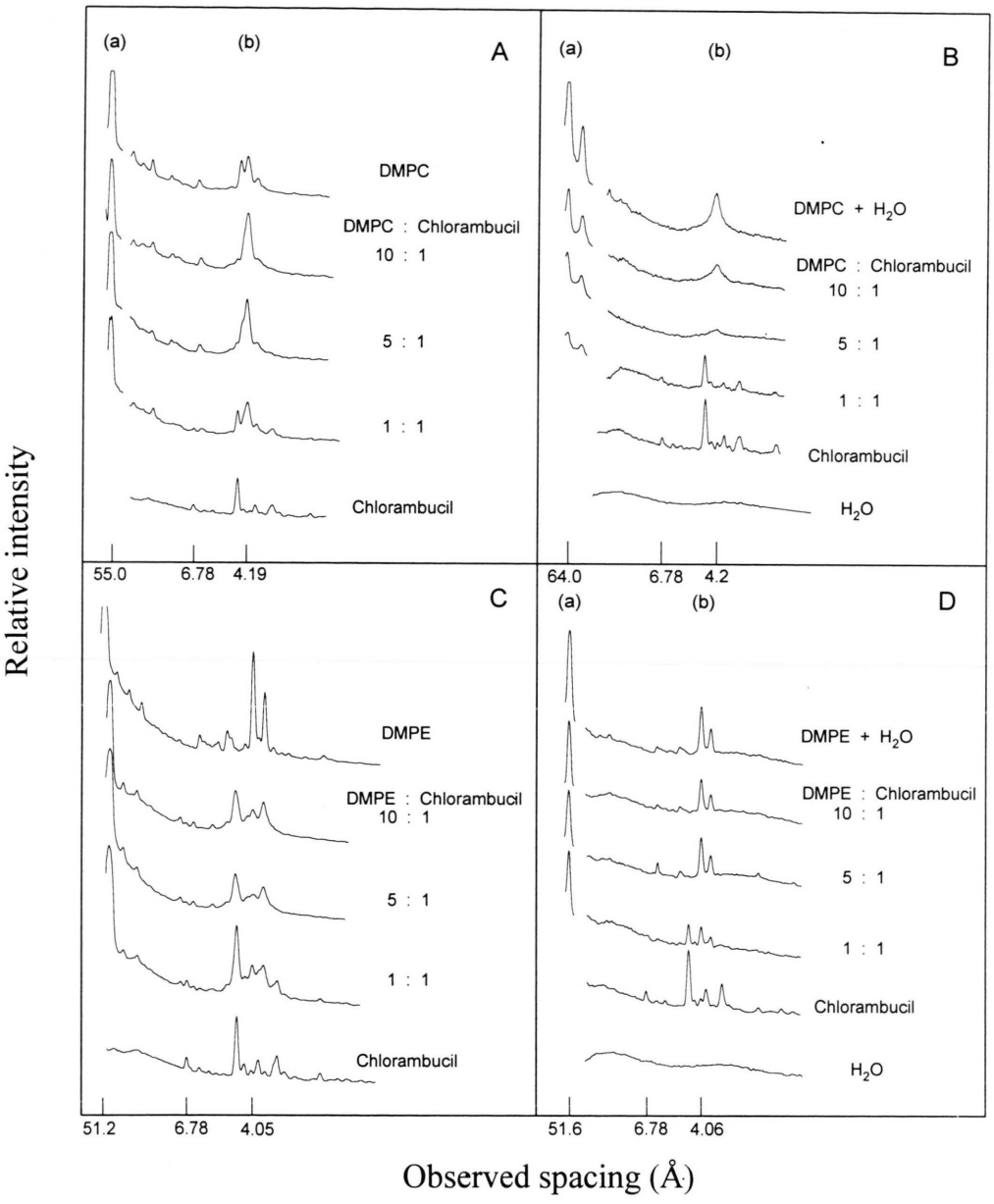

Fig. 3. Microdensitograms from X-ray diagrams of dimyristoylphosphatidylcholine (DMPC), dimyristoylphosphatidylethanolamine (DMPE) and of their 10:1, 5:1 and 1:1 molar mixtures with chlorambucil. Flat-plate cameras; specimen-to film distances: (a) 14 cm, (b) 8 cm; A and C: recrystallized from $CHCl_3$:CH_3OH 3:1 (v/v); B and D: immersed in water. The DMPE:chlorambucil mixtures show the pattern of DMPE Lc_1 form in D while that of its Lc_2 form is present in C.

bon chain packing order. Therefore, the decrease of this parameter can be explained by a structural perturbation of the bilayer hydrophobic region due to the incorporation of chlorambucil. On the other hand, the effect of laurdan GP indicated that the dynamics of the dipolar relaxation and/or the water penetration into the polar head group region was increased by the incorporation of the

Table I. Effect of chlorambucil on the anisotropy (r) of 1,6-diphenyl-1,3,5-hexatriene (DPH) and the general polarization (GP) of laurdan embedded in large unilamellar dimyristoylphosphatidylcholine (DMPC) vesicles (probe:lipid ratio 1:600).

Chlorambucil Conc. [mM]	r DPH	GP Laurdan
0.00	0.332	0.320
0.01	0.339	0.318
0.10	0.334	0.302
1.00	0.278	0.225

Each result represents the average of data in duplicate samples; between 6 and 12 determinations were performed in each sample. Mean standard deviation = 0.003.

drug. These results tend to agree with those observed in the X-ray diffraction experiments performed on DMPC multilayers in an aqueous medium.

Discussion

There is general consensus on the role of cell membranes in carcinogenesis (Galeotti *et al.*, 1988). Thus, the plasma membrane has gained increasing attention as a possible target of antitumor drugs (Luxo *et al.*, 1996; Speelmans *et al.*, 1996; Marutaka *et al.*, 1994). It has been reported that they act as growth factor antagonists, growth factor receptor blockers, interfere with mitogenic signal transduction or exert direct cytotoxic effects (Grunicke and Hoffmann, 1992). Moreover, drugs that interact with DNA cause structural and functional membrane alterations which may be essential for growth inhibition by these agents (Grunicke *et al.*, 1985b). Chlorambucil is an alkylating agent widely used in the treatment of chronic lymphocytic leukaemia. Contradictory reports have been published concerning its interaction with cell membranes. Thus, Grunicke *et al.* (1983) reported that it decreased fluidity of Ehrlich ascite tumor cells; after additional studies, they informed that no evidence was found for a change in membrane lipid fluidity (Grunicke *et al.*, 1985b). It has also been stated that alkylating agents had no effects in these systems even at highly toxic concentrations (Ankel *et al.*, 1986). Our results indicate that chlorambucil interacts with the erythrocyte membrane and phospholipid bilayers of molecular models. In fact, human erythrocytes incubated with a dose of chlorambucil equivalent to its therapeutical concentration in plasma, developed cup-shaped forms (stomatocytes). According to the bilayer couple hypothesis (Sheetz and Singer, 1974), the shape changes induced by foreign molecules in erythrocytes are due to a differential expansion of the two monolayers. Thus, stomatocytes are produced when the added compound locates into the inner leaflet whereas spiculated-shaped equinocytes are observed when it inserts into the outer moiety (Lange *et al.*, 1982). It can therefore be concluded that chlorambucil preferentially inserted into the inner monolayer of the erythrocyte membrane. This conclusion is supported by the results obtained with multilayers of DMPC and DMPE. X-ray diffraction analysis indicated that the drug interacted with both lipids. Chemically they only differ in their terminal amino groups, these being $^{+}NH_3$ in DMPE and $^{+}N(CH_3)_3$ in DMPC. 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 which is not significantly perturbed by water. On the other hand, the gradual hydration of DMPC bilayers leads to water filling the highly polar interbilayer spaces (Suwalsky, 1988). Consequently, there is a) an increase in its bilayer width from 55.0 Å when dry up to 64.0 Å when fully hydrated, and b) a decrease in the number of reflections due to the resulting increase in its fluidity. These conditions facilitate the incorporation of chlorambucil into DMPC bilayers, its penetration into the acyl chain and polar regions and the ensuing molecular perturbation of the phospholipid bilayer structure. Thus, apparently it is amazing that chlorambucil is able to induce a somewhat higher perturbation in the tighter DMPE structure than in the more open structure of DMPC. Although this effect is rather uncommon, it has been observed with other molecules (Suwalsky *et al.*, 1998; Suwalsky *et al.*, 1999). On the other hand, the results obtained by fluorescence spectroscopy in DMPC LUV tend to agree with those revealed by X-ray diffraction in DMPC multilayers. In conclusion, our results confirm that chlorambucil inserts

into the inner monolayer of the erythrocyte membrane. Although it cannot be ruled out its interaction with proteins, our results unambigously showed that chlorambucil interacted with lipid bilayers. Therefore, cell membrane structure and physiological properties such as fluidity, permeability, receptor and channel functions may be affected. Such interactivity of chlorambucil with the cell membrane might explain its cytotoxic effect.

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McEnvoy G. K., (ed.), 1995, American Hospital Formulary Service Drug Information 95, 36th ed., pp. 607–611, American Society of Hospital Pharmacists, Inc., USA.

Ankel E. G., Ring B. J., Lai C. S. and Holcenberg J. S. (1986), The lack of effects of alkylating agents on mammalian cell membranes. Int. J. Tissue React. **8**, 347–354.

Devaux P. F. and Zachowsky A. (1994), Maintenance and consequences of membrane phospholipid asymmetry. Chem. Phys. Lipids **73**, 107–120.

Galeotti T., Cittadini A., Neri G. and Scarpa A.(1988), In: Membrane in Cancer Cells (Galeotti T., Cittadini A., Neri G. and Scarpa A., eds.), Ann. New York Acad. Sci. **551**, xiii-xiv.

Grunicke H., Grünewald K., Helliger W., Scheidl F., Wolff-Schreiner E. and Puschendorf B. (1983), Inhibition of tumor growth by an alkylation of the plasma membrane. Adv. Enzyme Regul. **21**, 21–30.

Grunicke H., Doppler W., Hoffmann J., Lindner H., Maly K., Oberhuber H., Ringsdorf H. and Roberts J. J. (1985a), Plasma membrane as target of alkylating agents. Adv. Enzyme Regul. **24**, 247–261.

Grunicke H., Doppler S. A., Finch S. A. E., Greinert R., Grünewald K., Hofmann J., Maly K., Stier A., Scheidl F. And Thomas J. K. (1985b), Effects of nitrogen mustard on potassium transport systems and membrane structure of Ehrlich ascites tumor cells. Adv. Enzyme Regul. **23**, 277–290.

Grunicke H. and Hoffmann J. (1992), Cytotoxic and cytostatic effects of antitumor agents induced at the plasma membrane level. Pharmacol. Ther. **55**, 1–30.

Lange Y., Gough A. and Steck T. L. (1982), Role of the bilayer in the shape of the isolated erythrocyte membrane. J. Membr. Biol. **69**, 113–123.

Löf K., Hovinen J., Reinikainen P., Vilpo L. M., Seppälä E., Vilpo J. A. (1997), Kinetics of chlorambucil in vitro: effects of fluid matrix, human gastric juice, plasma proteins and red cells. Chem-Biol. Inter. **103**, 187–198.

Luxo C, Jurado A. S., Custodio J. B. A. and Madeira V. M. (1996), Use of *Bacillus stearothermphilus* as a model to study tamoxifen-membrane interactions, Toxicol. in Vitro **10**, 463–471.

Marutaka M., Iwagachi H., Suguri T., Tanaka N. And Orita K. (1994), Alterations of membrane fluidity in K562 cells exposed to the anticancer drug adriamycin. Res. Com. Mol. Pathol. Pharmacol. **85**, 163–170.

Parasassi T. and Gratton E. (1995), Membrane lipid domains and dynamics as detected by laurdan fluorescence **5**, 59–69.

Povirk L. F. and Shuker D. E. (1994), DNA damage and mutagenesis induced by nitrogen mustards. Mutat. Res. **318**, 205–226.

Sheetz M. P. and Singer S. J. (1974), Biological membranes as bilayer couples. A molecular mechanism of drug induced interactions. Proc. Natl. Acad. Sci. USA **71**, 4457–4461.

Shulman L. N. (1993), The biology of alkylating-agent cellular injury. Hematol. Oncol. Clin. North Am. **7**, 325–335.

Speelmans G., Staffhorst R W. H. M., Steenbergen G. and de Kruijff B. (1996), Transport of the anti-cancer drug across cytoplasmic membranes and membranes composed of phospholipids derived from *Escherichia coli* occurs via a similar mechanism. Biochim. Biophys. Acta **1284**, 240–246.

Suwalsky M. (1988), In: Physical Properties of Biological Membranes and Their Functional Implications (Hidalgo, C., ed.), chapter 1. Plenum, USA.

Suwalsky M., Gimenez L., Saenger V. and Neira F. (1988), X-ray studies on phospholipid bilayers. VIII. Interactions with chlorpromazine. HCl. Z. Naturforsch. **43c**, 742–748.

Suwalsky M., Rodríguez C., Villena F., Aguilar F. and Sotomayor, C. P. (1998), The organochlorine pesticide lindane interacts with the human erythrocyte membrane. Pest. Biochem. Physiol. **62**, 87–95.

Suwalsky M., Hernández P., Villena F., Aguilar F. And Sotomayor C. P. (1998), Interaction of the anticancer drug tamoxifen interacts with the human erythrocyte membrane and molecular models. Z. Naturforsch. **53c**, 182–190.

Suwalsky M., Hernández P., Villena F., Aguilar F. And Sotomayor C. P. (1999), Interaction of the anticancer drug adriamycin interacts with the human erythrocyte membrane. Z. Naturforsch. **54c**, 271–277.

Suwalsky M., Rodríguez C., Villena F., Aguilar F. and Sotomayor C. P. (1999), The pesticide hexachlorobenzene induces alterations in the human erythrocyte membrane. Pest. Biochem. Physiol. **65** (in press).