Molecular Interactions of Quinidine with Phospholipid Bilayers

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Quinidine, Antiarrhythmic Drug, Phospholipid Bilayer, Liposomes, Erythrocyte Membrane

Quinidine (QUIN) is one of the most important and efficient antiarrhythmic drugs (AAD). It belongs to class I, which are the drugs that exert their action at the level of the sodium channels in the membrane of the myocard. Several hypotheses support the idea that the molecular mechanism of action of the AAD is via nonspecific interactions with phospholipids sited in the neighborhood of the channels. In order to probe the validity of these hypotheses, QUIN was made to interact with the phospholipids dimyristoylphosphadidylcholine (DMPC) and dimyristoylphosphatidylethanolamine (DMPE). These interactions were performed in a hydrophobic and a hydrophilic medium under a wide range of molar ratios. The resulting products were analyzed by X-ray diffraction. QUIN solutions were also made to interact with DMPC liposomes, which were studied by fluorescent spectroscopy. Finally, human erythrocytes which were incubated with QUIN solutions were observed by scanning electron microscopy. The results of these experiments proved that QUIN indeed interacted with phospholipid bilayers.

Introduction

Since its initial use quinidine (QUIN), whose structural formula is shown in Fig. 1, has remained as one of the most important and efficient drug for maintaining the heart rhythm (Levy and Azoulay, 1994). It has been classified into class I of the antiarrhythmic drugs (AAD) (Bigger and Hoffman, 1990), which are those that exert their action at the level of the sodium channels in the membrane of the myocard (Ravens, 1992). However, it has been reported that QUIN also inhibits the calcium channels (Nawada *et al.*, 1994). Several hypotheses have been proposed to explain the molecular mechanism of action of the AAD. Obviously, one them suppose that the AAD directly enter into the channels to block the passage of ions across the membrane (Katz *et al.*, 1982). However, other hypotheses support the idea that AAD interact in nonspecific ways with phospholipid bilayers located in the neighborhood or surrounding the ion channels (Katz *et al.*, 1982). Thus, the amphiphilic AAD would insert into the lipid layers producing lateral and/or longitudinal expansions which would result in a compression of the protein units that form the channels hindering the proper permeation of ions. A somewhat related mechanism, the "membrane bilayer hypothesis" (Hille, 1977) might also apply to AAD. Accordingly to it, certain hydrophobic and amphiphilic drugs may necessarily partition into the lipid bilayer of the membrane prior to protein receptor binding. The lipid bilayer may assist successful drug-receptor recognition and binding by optimizing the location, orientation and concentration of the drug molecule with respect to the receptor site (Mason, 1993).

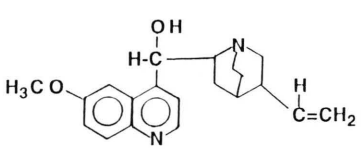

Fig. 1. Structural formula of quinidine (QUIN).

For these reasons it was thought of interest to study the interaction of QUIN with phospholipid bilayers. These interactions were performed in model and cell membranes by three different approaches. In the first, X-ray diffraction methods were used to follow the ability of QUIN to perturb the structure of dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylethanol-

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amine (DMPE) multibilayers. These are the types of phospholipids that are respectively found in the outer and inner monolayers of the erythrocyte membrane (Roelofsen, 1991). These interactions were performed in a hydrophobic and a hydrophilic medium given the amphiphilic nature of QUIN, DMPC and DMPE. Fluorescence spectroscopy on DMPC large unilamellar vesicles was the second approach. The fluorescence steady-state anisotropy of 1,6-diphenyl-1,3,5-hexatriene (DPH) and the general polarization of 6-dodecanoyl-2-dimethylaminonaphtalene (Laurdan) were determined for different concentrations of QUIN. The third approach consisted in incubating QUIN, at the same molar concentrations as in the other two methods, with human erythrocytes. The red cells were later observed by scanning electron microscopy (SEM) to detect the shape changes induced by QUIN.

Materials and Methods

Synthetic DMPE from Sigma, USA (Lots 67F-8350 and 13H-83681, A grade, MW 635.9), DMPC from Sigma, USA (Lots 57F-8365 and 80H-8371, A grade, MW 677.9) and quinidine sulphate, MW 783.0 (a gift from Drs. R. Mannhold and W. Voigt, University of Düsseldorf, Germany) were used without further purification. About 3 mg of each phospholipid were mixed with the corresponding weight of QUIN in order to attain DMPC:QUIN and DMPE:QUIN 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 very carefully. The resulting samples, under the form of crystalline powders, were introduced into special glass capillaries 0.7 mm diameter. They were X-ray diffracted in Debye-Scherrer cameras of 114.6 mm diameter and flat-plate cameras with 0.25 mm dia. glass collimators (Suwalsky, 1988), provided with rotating devices. The same procedure was also followed with samples of each phospholipid and QUIN. The hydrated specimens were prepared in 1.5 mm dia. glass capillaries, each containing 2–3 mg of DMPC or DMPE. Each capillary was then filled with about 100 µl of a) distilled water, b) 0.01 mM, c) 0.1 mM, d) 1.0 mM and e) 10 mM QUIN aqueous solutions. They were X-ray diffracted 2 and 14 days after preparation in flat-plate cameras. Specimen-to-film distances were 8 or 14 cm, standardized by sprinkling calcite powder on the capillary surface. Ni-filtered CuKα radiation from a Philips PW 1140 X-ray generator was used. The relative reflection intensities were obtained from films by peak-integration in a Joyce-Loebl MKIIICS microdensitometer connected to an Acer 915 computer. No correction factors were applied. The experiments with aqueous solutions were performed at 17 ± 2 °C, which is below the main transition temperatures of both DMPC and DMPE.

The influence of QUIN in the physical properties of DMPC large unilamellar vesicles was studied by fluorescence spectroscopy using DPH and Laurdan as fluorescent probes. DPH is widely used as a probe for the hydrophobic regions of the phospholipid bilayers because of its favorable spectral properties. Its fluorescence steady-state anisotropy measurements were used to investigate the structural and dynamic properties of DMPC as it provided a measure of the rotational diffusion of the fluorophor, restricted within a certain region such as a cone, due to the lipid hydrocarbon chain packing order. On the other hand, 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 provided information of the dynamic properties in the zone of DMPC liposomes which was related to the lipid polar group organization. The quantitation of Laurdan fluorescence spectra intensity data at different wavelengths was done using the general polarization (GP) concept (Parasassi *et al.*, 1990). A detailed description of the techniques and instruments employed have already been described elsewhere (Suwalsky *et al.*, 1994).

The interaction with human erythrocytes was performed by incubating QUIN with blood samples from clinically healthy individuals not being under treatment with any pharmacological drug. For this purpose, a blood stock solution (BSS) was prepared by mixing one drop of blood with 5 ml of phosphate buffer saline (PBS) at 5 °C. From this BSS solution the following samples were prepared: a) control, by mixing 9 ml of PBS with 1 ml of blood stock solution, and b) 0.01 mM, 0.1 mM and 1 mM QUIN solutions by the adequate mixture of BSS with QUIN containing PBS solution.

These samples were incubated at 37 °C for 1 hour and fixed with glutaraldehyde. Drops of the fixed samples on Al stubs were dried at 37 °C for 1 hour and gold coated for 3 min at 10^{-1} Torr, reaching a final film width of 35–40 nm. The samples thus prepared were observed and photographed in a Etec Autoscan SEM.

Results

X-ray studies on lipid multilayers

The molecular interactions of QUIN with DMPC and DMPE were studied in a hydrophobic and hidrophilic medium given their amphiphilic nature. Table I shows the interplanar spacings and relative intensities of the reflections produced by DMPC, QUIN and their 10:1, 5:1 and 1:1 molar mixtures after interacting and being recrystallized from chloroform:methanol 3:1 v/v solutions. Their respective diffractograms are compared in Fig. 2. The perturbing effect of QUIN upon DMPC structure was very mild. In fact, there was only a slight increase of the lipid bilayer width from 54.5 Å up to 55.9 Å and a decrease of DMPC intensities. The results from their interactions in an aqueous medium are presented in Table II and Fig. 3. When exposed to pure water DMPC expanded its bilayer width from 54.5 Å to 65.5 Å. At the same time, the observed reflections were reduced to only the first three orders of the bilayer repeat and one of

Table I. Comparison of observed interplanar spacings (do) and relative intensities (Io rel) of DMPC, QUIN and their 10:1, 5:1 and 1:1 molar mixtures[a,b,c].

DMPC		DMPC:QUIN 10:1		DMPC:QUIN 5:1		DMPC:QUIN 1:1		QUIN	
do [Å]	Io rel	do [Å]	Io rel	do [Å]	Io rel	do [Å]	Io rel	do [Å]	Io rel
54.5*	349	54.5*	435	55.2*	575	55.9*	244	–	–
27.3	2	27.3	2	27.4	3	27.8	1	–	–
18.3	2	18.3	1	18.2	1	18.6	1	–	–
13.6	9	13.6	7	13.6	7	13.6	6	–	–
9.21	6	9.27	4	9.25	3	9.34	4	9.35	3
8.25	5	8.22	3	8.22	2	–	–	–	–
6.25	14	6.25	8	6.27	5	–	–	–	–
5.65	3	5.68	2	5.65	1	–	–	–	–
–	–	–	–	–	–	5.41	14	5.36	21
4.70	14	4.65	8	4.67	8	4.73	5	4.74	6
4.29	45	4.29	29	4.29	19	4.30	13	–	–
4.11	100	4.12	68	4.12	57	4.11	28	4.09	11
3.88	44	3.85	20	3.85	17	3.82	11	3.80	8
3.66	7	3.66	5	3.67	5	3.73	4	3.70	11

[a] All the specimens were recrystallized from $CHCl_3$:CH_3OH 3:1.
[b] The interplanar spacings and intensities of the reflections were measured in X-ray diagrams obtained from Debye-Scherrer and flat-plate cameras with 8 and 14* cm specimen-to-film distances.
[c] Only the main observed reflections are included.

Table II. Comparison of observed interplanar spacings (do) and relative intensities (Io rel) of DMPC specimens with water and QUIN aqueous solutions[a,b].

DMPC + H_2O		DMPC + 0.01 mM QUIN		DMPC + 0.1 mM QUIN		DMPC + 1 mM QUIN		DMPC + 10 mM QUIN	
do [Å]	Io rel	do [Å]	Io rel	do [Å]	Io rel	do [Å]	Io rel	do [Å]	Io rel
65.5*	160*	64.0*	74*	63.3*	42*	–	–	–	–
32.5*	100*	32.0*	58*	31.8*	34*	–	–	–	
21.3	6	21.3	2	–	–	–	–	–	–
4.19	57	4.20	44	4.20	7	4.20	9	–	–

[a] The interplanar spacings and intensities of the reflections were measured in X-ray diagrams obtained from flat-plate cameras with 8 and 14* cm specimen-to-film distances.
[b] The samples were diffracted 2 and 14 days after preparation. No differences were observed.

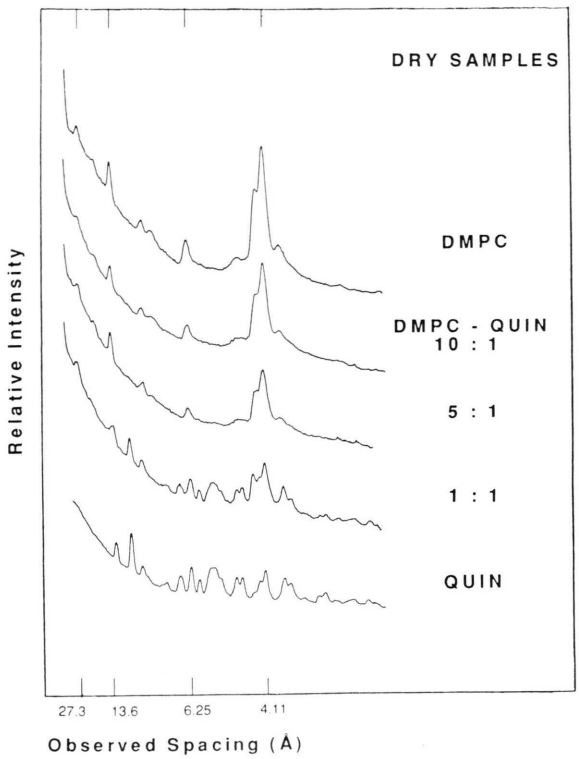

Fig. 2. Microdensitograms from X-ray diagrams of specimens recrystallized from $CHCl_3$:CH_3OH 3:1 (v/v). Flat-plate cameras. Specimen-to-film distance 8 cm.

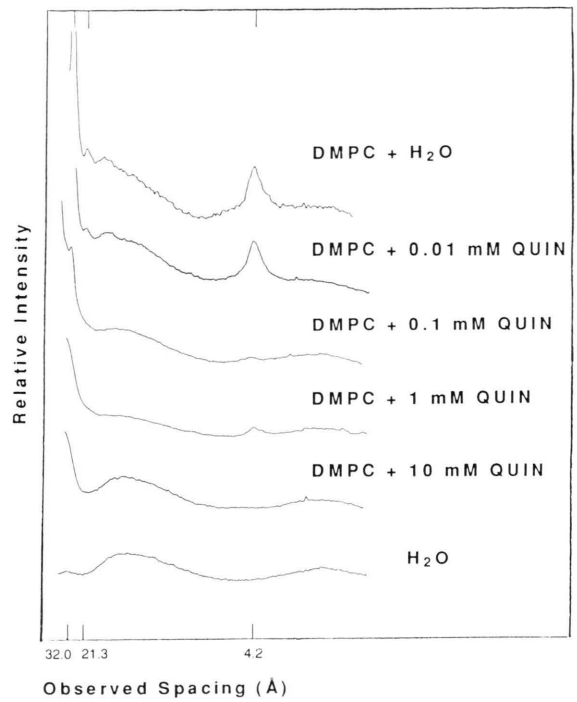

Fig. 3. Microdensitograms from X-ray diffraction diagrams of aqueous mixtures of DMPC. Flat-plate cameras. Specimen-to-film distance 8 cm.

4.2 Å. About the same X-ray pattern remained when DMPC was allowed to interact with 0.01 mM and 0.1 mM QUIN solutions, although the latter produced a considerable weakening of the lipid reflections. When QUIN concentration was increased to 1.0 mM only remained the 4.2 Å reflection, which also disappeared in a 10 mM QUIN solution. As it can be observed in Fig. 3, this pattern looks very much alike to that of pure water.

The results of the interactions of QUIN with DMPE in the hydrophobic medium are shown in Table III and Fig. 4. As it has been reported elsewhere (Suwalsky *et al.*, 1990), DMPE presents two polymorphic forms when it is recrystallized from chloroform:methanol. One phase, Lc_1, is obtained when these solvents are in a 3:1 v/v ratio and it is characterized because the hydrocarbon chains are extended and parallel to the bilayer normal. The Lc_2 phase, obtained from a 1:3 solvent ratio, presents the hydrocarbon chains tilted by about 30° and a bilayer width of nearly 44 Å compared with the about 52 Å of the Lc_1 form. QUIN produced the following effects: a) DMPE underwent a Lc_1 to Lc_2 phase transition when its molar ratio with QUIN was 10:1; its bilayer width increased slightly while the reflection intensities decreased; b) higher proportions of QUIN produced about the same patterns than that of the 10:1 ratio; c) QUIN reflections were present in all its mixtures. These results clearly indicate that QUIN interactions with DMPE in the hydrophobic medium were very mild. Finally, as it can be observed in Table IV and Fig. 5, water did not affect the bilayer structure of DMPE. However, QUIN increased its bilayer width from about 51 Å to nearly 56 Å and decreased the intensities of the 4.04 Å and 3.78 Å reflections.

Fluorescence studies on vesicles

The interaction of QUIN with DMPC large unilamellar vesicles was studied evaluating DPH steady state anisotropy and Laurdan general polarization. The results are presented in Table V.

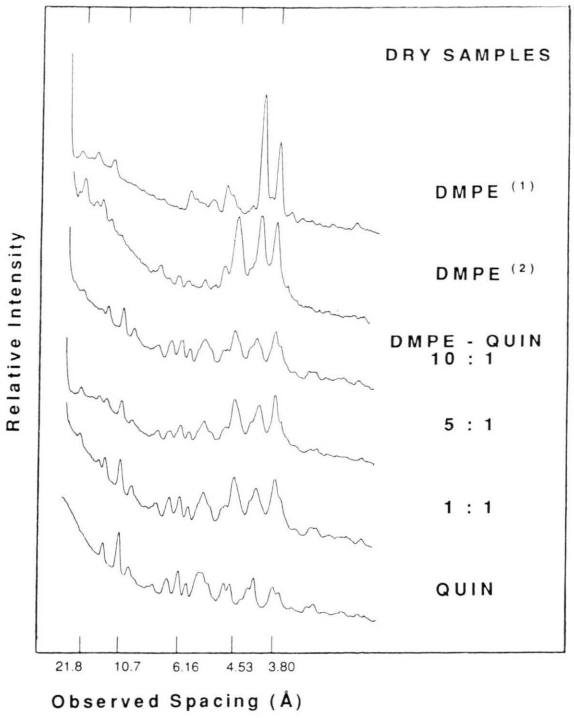

Fig. 4. Microdensitograms from X-ray diagrams of specimens recrystallized from $CHCl_3$:CH_3OH 3:1 (v/v). Flat-plate cameras. Specimen-to-film distance 8 cm. 1) Phase Lc_1, 2) Phase Lc_2.

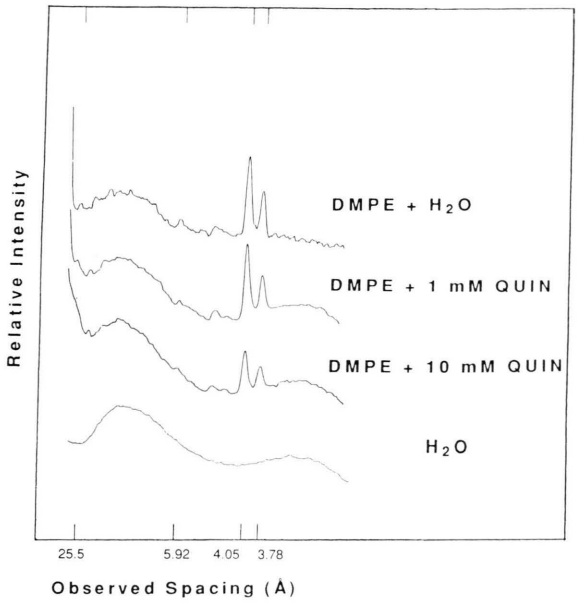

Fig. 5. Microdensitograms from X-ray diffraction diagrams of aqueous mixtures of DMPE. Flat-plate cameras. Specimen-to-film distance 8 cm.

The presence of increasing concentrations of QUIN up to 10 mM produced a monotonous decrease in the fluorescence anisotropy of DPH and only a slight decrease in the general polarization of Laurdan. These results indicate that QUIN has a significant influence on the rotational mobility of DPH, distributed in the hydrophobic zone of the lamella, but not in the environment of Laurdan at the lamella hydrophilic- hydrophobic interface.

Morphological studies on erythrocytes

QUIN was made to interact *in vitro* with human erythrocytes in final concentrations that ranged between 0.01 m_m, which is about its therapeutical plasma concentration (Mannhold *et al.*, 1990) and 10 mM. The observations made by SEM indicated that 0.01 mM QUIN did not produce any noticeable change to the erythrocyte shape. However, higher concentrations produced a deepening of the central concavity of the cells, which took a cup-shaped form known as stomatocyte. QUIN did not produce lysis in any of the assayed concentrations.

Discussion

The experimental results described above clearly indicate that QUIN interacted with both phospholipid bilayers. However, the type and extent of these interactions depended on the nature of the phospholipids involved, their phase state and concentration ratio with QUIN. In fact, the X-ray studies showed that QUIN interacted with DMPC bilayers in both a hydrophobic and a hydrophilic medium. This is not surprising given the open packing of DMPC bilayers that allows water and amphiphilic molecules to incorporate and perturb its molecular arrangement. DMPE, on the contrary, presents such a close and tight molecular packing that it is very difficult for foreign molecules to penetrate and affect its structure (Suwalsky, 1988). Therefore, the observed effects of QUIN upom DMPE bilayer structure were quite unexpected.

The spectroscopic results obtained from the interaction of QUIN with DMPC liposomes mostly agree with those observed by X-ray diffraction on

DMPC multilayers in a hydrophilic medium. In fact, the rotational behavior of DPH in an anisotropic system such as the bilayer is considered an hindered rotation. In this model, the steady state anisotropy can be mainly related to the restriction of the rotational motion due to the hydrocarbon packing order (Weber, 1978; Kawato *et al.*, 1977). In this context, DPH fluorescence anisotropy decrease can be rationalized as a perturbation of DMPC hydrocarbon chains, as it was observed in the X-ray experiments. Laurdan, on the other hand, has been reported to be located at the hydrophobic-hydrophilic interface of the lamella (Chong, 1988; Chong, 1990). Its spectral sensitivity to the phospholipid phase state has been attributed to a dipolar relaxation phenomena originated from the sensitivity of the probe to the polarity of its environment. This is due to the large charge separation that the fluorophor exhibits upon excitation, i.e., a large excited state dipole (Parassasi *et al.*, 1986). In a phospholipid bilayer, the relaxation process depends on the dynamics of the dipole moieties surrounding the Laurdan excited state molecules at this interface (Parassasi *et al.*, 1990). Accordingly to the results shown in Table V, the dynamics of Laurdan environment, i.e., the phospholipid polar groups and water molecules, were not significatively perturbed by QUIN. This result differed from that obtained in the X-ray experiments, in which QUIN produced a perturbation of DMPC polar heads. This is probably due to the difference of the physical process involved in these measurements, which are related to the dynamics of molecular events in comparison with the static character of the X-ray information.

Table III. Comparison of observed interplanar spacings (do) and relative intensities (Io rel) of DMPE, QUIN and their 10:1, 5:1 and 1:1 molar mixtures[a,b,c].

DMPE(1)		DMPE(2)		DMPE:QUIN 10:1		DMPE:QUIN 5:1		DMPE:QUIN 1:1		QUIN	
do [Å]	Io rel	do [Å]	Io rel	do [Å]	Io rel	do [Å]	Io rel	do [Å]	Io rel	do [Å]	Io rel
50.8*	595*	–	–	–	–	–	–	–	–	–	–
–	–	44.1	1130	44.6	412	44.6	427	44.6	300	–	–
25.4	6	–	–	–	–	–	–	–	–	–	–
–	–	21.8	7	21.8	3	22.4	4	22.2	4	–	–
17.1	7	17.3	3	–	–	–	–	–	–	–	–
–	–	14.7	6	14.9	3	14.9	3	14.7	3	–	–
–	–	–	–	13.1	8	13.1	11	13.1	11	13.1	11
12.7	7	12.7	3	–	–	–	–	–	–	–	–
–	–	11.1	1	10.7	15	10.7	12	10.7	21	10.7	22
–	–	–	–	6.16	19	6.16	14	6.16	19	6.15	15
5.65	10	–	–	–	–	–	–	–	–	–	–
–	–	5.32	4	5.27	37	5.31	25	5.29	30	5.28	38
4.77	22	4.80	11	4.81	7	4.81	7	4.81	7	4.80	4
–	–	–	–	4.71	7	4.72	6	4.70	6	4.74	11
–	–	4.52	36	4.53	41	4.54	58	4.53	66	4.55	15
–	–	4.40	19	4.42	14	4.43	8	4.41	14	4.36	3
4.22	3	4.19	8	4.21	5	4.23	11	4.19	11	4.20	10
4.05	100	4.04	55	4.08	55	4.07	58	4.07	60	4.09	34
3.80	53	3.81	42	3.80	48	3.81	52	3.82	52	3.80	14
–	–	–	–	3.70	16	3.72	15	3.71	21	3.70	19

[a] All the specimens were recrystallized from $CHCl_3$:CH_3OH 3:1.
[b] The interplanar spacings and intensities of the reflections were measured in X-ray diagrams obtained from Debye-Scherrer and flat-plate cameras with 8 and 14* cm specimen-to-film distances.
[c] Only the main observed reflections are included.
(1) Phase Lc_1.
(2) Phase Lc_2.

The shape change produced to human erythrocytes might be another indication of QUIN interaction with phospholipid bilayers. Several studies have shown that amphiphilic drugs induce a transformation from the discoid shape of the red cells to spiculated (echinocyte) or cupped (stomatocyte) forms (Fujii *et al.*, 1979; Lange *et al.*, 1982; Nwafor and Coackley, 1985; Isomaa *et al.*, 1987). Sheetz and Singer (1974, 1976, 1977) formulated

Table IV. Comparison of observed interplanar spacings (do) and relative intensities (Io rel) of DMPE specimens with water and QUIN aqueous solutions[a,b].

DMPE + H_2O		DMPE + 1 mM QUIN		DMPE + 10 mM QUIN	
do [Å]	Io rel	do [Å]	Io rel	do [Å]	Io rel
50.2*	526*	55.9*	505*	55.9*	506*
25.5*	2	28.0*	3*	–	–
17.1	6	18.6	6	18.6	2
14.5	2	16.1	2	15.9	2
12.8	9	14.1	8	14.1	3
11.1	4	–	–	–	–
10.4	2	–	–	–	–
7.20	3	7.98	2	8.04	2
5.93	5	5.92	3	5.91	3
5.63	4	5.57	3	5.56	4
5.40	3	5.33	2	5.32	2
5.07	7	5.09	2	5.07	1
4.78	18	4.77	13	4.77	8
4.63	6	4.66	5	–	–
4.47	3	4.47	2	4.46	5
4.22	3	–	–	–	–
4.04	100	4.05	89	4.05	55
3.90	8	–	–	–	–
3.78	63	3.79	45	3.78	27
3.64	5	–	–	–	–
3.51	2	–	–	–	–
3.38	3	–	–	–	–
3.15	3	–	–	–	–

[a] The interplanar spacings and intensities of the reflections were measured in X-ray diagrams obtained from flat-plate cameras with 8 and 14* cm specimen-to-film distances.

[b] The samples were diffracted 2 and 14 days after preparation. No differences were observed.

Table V. Effect of quinidine (QUIN) 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.

QUIN Conc.	r DPH	GP Laurdan
0.0 mM	0.31	0.54
0.04 mM	0.27	0.55
0.5 mM	0.12	0.55
8.5 mM	0.01	0.49

the "bilayer couple hypothesis" to explain these shape alterations. Accordingly to it, they would arise from a differential expansion of the two lipid monolayers of the erythrocyte membrane. Thus, the stomatocytic shape of the erythrocytes in vitro would indicate that QUIN intercalated in the inner monolayer of the cell membrane. Similar results have been reported for the AAD amiodarone (Reinhart and Rohner, 1990). On the basis of the X-ray and spectroscopic results either an echinocytic or a stomatocytic form would have been expected given QUIN interactions with both DMPC and DMPE. However, accordingly to Reinhart and Rohner (1990), the stomatocytic form is attained at low cholesterol/phospholipid ratio, which is the case of normal erythrocytes.

In conclusion, different methods confirmed the fact that QUIN interacts with phospholipid bilayers. Therefore, the suggested molecular mechanisms of action of class I AAD, involving the lipidic moiety of cell membranes, might be valid.

Acknowledgements

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