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Structure of the adriamycin-cardiolipin complex

Role in mitochondrial toxicity

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Adriamycin and its derivatives are among the most efficient antimitotics used in clinical therapy. A specific cardiotoxicity places a limit on the total dose of adriamycin that may be administered. The mechanism of cardiac toxicity is complex. Data accumulated from in vitro and in vivo studies indicate a possible common cause for the inhibition of numerous enzymes and tissue degradation by a free radical mechanism: the binding of adriamycin to the inner mitochondrial membrane cardiolipin. The structure of the adriamycin-cardiolipin complex has been investigated by using physico-chemical techniques and via conformational analysis. The results open a rational way to design new structures that are less cardiotoxic.

1. Introduction

Adriamycin (ADM) is one of the most effective agents against leukemia and solid tumors. Its mode of interaction with the nuclear target has been extensively reviewed [1] and is assumed to be responsible for its antimitotic activity. Both X-ray measurements and conformational analysis indicate that the planar moiety of adriamycin intercalates between the base-pairs of DNA, whereas the sugar moiety fits into the double helix minor groove. Adriamycin exerts toxic side effects on a large variety of cells. Its cardiotoxicity, however, places a limit on the total dose that may be given. Interestingly, in a series of related anthracycline glycoside drugs, dose-limiting cardiac toxicity can be dissociated from the antitumor activity, suggesting distinct modes of action [2]. Much evidence indicates that the mitochondrial membrane could be the target responsible for cardiac toxicity; indeed, the development of cardiac failure induced by adriamycin is correlated with the impairment of mitochondrial functions such as O_2 consumption and ATP synthesis. Rhythmic contractions characteristic of myocardial cells in culture cease with adriamycin treatment [3–5] concomitant with a significant decrease in ATP and phosphocreatine concentrations [6].

Our main objective is to describe the mitochondrial sites of adriamycin binding. It is unlikely that adriamycin interacts with each inhibited enzyme along the electron-transport chain between NADH and O_2 in order to cause the multiple effects observed on the whole respiratory chain [7]. Interaction of the drug with a unique

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phospholipid site could account for all of the observed effects. Cardiolipin, a phospholipid specific to the inner mitochondrial membrane, has been suggested to play this role [8]. Enzymes of the respiratory chain require cardiolipin for full activity [9,10]. Among a large number of derivatives, adriamycin, which is the most cardiotoxic compound known, forms the strongest complex with cardiolipin [11]. Weak toxicity of other derivatives at this level can be correlated to a relatively weak affinity for cardiolipin. For example, rubidazone, which is less toxic than adriamycin at the mitochondrial level [12], binds less effectively to cardiolipin. Finally, *N*-acetyladriamycin, which does not bind to cardiolipin, perturbs neither rat electrocardiograms nor mitochondrial respiration [13]. The present article was prompted by our goals of elucidating the nature of the drug-cardiolipin complex and establishing the manner in which it participates in the phenomenon of mitochondrial cardiotoxicity.

2. Involvement of cardiolipin-adriamycin complex formation in mitochondrial enzyme activity

Two major modes of toxicity of ADM and its derivatives at the level of the inner mitochondrial membrane have been identified. The first involves specific molecular interactions between adriamycin and cardiolipin while the second implies free radical formation at the level of the anthraquinone group of ADM. Both require the initial binding of adriamycin to cardiolipin, and are briefly reviewed below.

2.1. Inhibition of inner mitochondrial membrane enzymes (complex IV and complex I–III)

Our group has mainly investigated the adriamycin-induced modification of cytochrome *c* oxidase and complex I–III activity. An absolute requirement of cardiolipin for the final oxidation site along the respiratory chain has been demonstrated [9], and only the number of cardiolipin molecules associated with the cytochrome *c* oxidase remains under discussion. Lipid-enzyme interactions are clearly of prime importance for cytochrome *c* oxidase activity. The mechanism of inhibition of cytochrome *c* oxidase activity by seven anthracycline glycosides [14] was shown to result from complexation of the enzyme-cardiolipin environment rather than from direct interaction between drug and enzyme [14]. Fig. 1 shows the linear relationship found between the affinity of the drug for cardiolipin and the drug concentration inhibiting 50% of the cytochrome *c* oxidase activity in mitochondria extracted from bovine heart. Moreover, the same drug (namely, adriamycin) inhibits the enzymatic activity to a different extent if purified and lipid-depleted cytochrome *c* oxidase is reactivated in proteoliposomes by cardiolipin or phosphatidic acid. (Phosphatidic acid is found in very small amounts in the mitochondrial membrane but is able to reactivate cardiolipin-depleted cytochrome *c* oxidase in vitro.) The affinity of adriamycin for cardiolipin is about 80-times higher than that for phos-

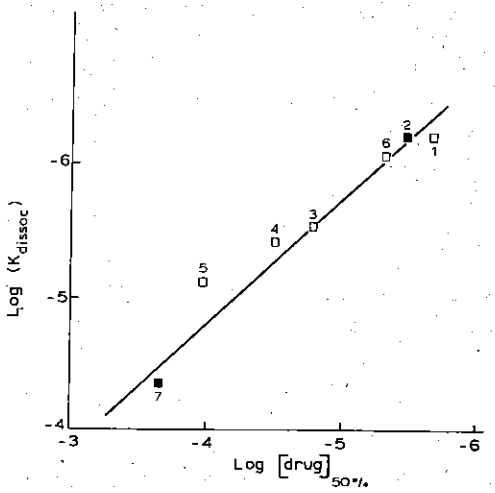

Fig. 1. Relation between the anthracycline glycoside concentration inhibiting 50% of the cytochrome *c* oxidase activity in isolated bovine heart mitochondria and the dissociation constant of the cardiolipin-drug complex. 1, adriamycin; 2, cinerubin; 3, rubidazone; 4, nogalamycin; 5, rhodomycin. Relation between adriamycin concentration inhibiting 50% of cytochrome *c* oxidase activity in a system containing purified and lipid-depleted cytochrome *c* oxidase included in pure cardiolipin liposomes (6) or pure phosphatidic acid liposomes (7) and its affinity for cardiolipin and phosphatidic acid, respectively.

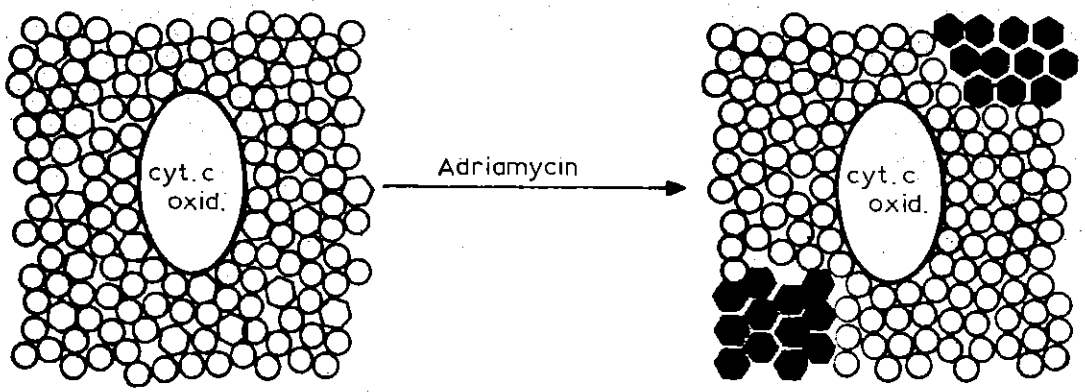

Fig. 2. Schematic representation of the mechanism of inactivation of cytochrome *c* oxidase. (Left) Cardiolipin (○) is in close contact with the enzyme, permitting its activity. After reaction with adriamycin (right), the complexed cardiolipin (○) segregates into a separate phase that is inaccessible to the enzyme, which remains in a lipid environment (○) incapable of activating it.

phatidic acid [8] and the adriamycin concentration required to inhibit 50% of the cytochrome *c* oxidase activity is precisely 80-times higher in the phosphatidic acid-reconstituted system than that in the case of cardiolipin. Similarly, Mende et al. [15] and Cheneval et al. [16] were recently able to demonstrate that purified mitochondrial phosphate carrier is activated by cardiolipin and inhibited by adriamycin. Differential scanning calorimetry measurements carried out on mixed dipalmitoylphosphatidylcholine (DPPC)-cardiolipin liposomes demonstrated that, following addition of adriamycin, the adriamycin-cardiolipin complex segregates in the lipid matrix to form a separate phase [16]. This model may be tentatively extended to the proteoliposome system containing cardiolipin, various phospholipids and cytochrome *c* oxidase. A schematic representation of the hypothetical enzyme inactivation mechanisms is proposed in fig. 2. The mechanism of interaction between cytochrome *c* and cytochrome *c* oxidase suggests another possibility for explaining the inhibition of cytochrome *c* oxidase due to the formation of the cardiolipin-drug complex. Cytochrome *c* is believed to bind to cardiolipin and to induce cardiolipin non-bilayer structures in order to reach a region of the cytochrome *c* oxidase complex buried within the bilayer [17]. ^{31}P-NMR measurements showed that adriamycin indeed inhibits formation of the non-bilayer cardiolipin structures [18] normally induced by the presence of cytochrome *c*.

Complex I–III of the mitochondrial membrane (NADH:cytochrome *c* oxidoreductase) is also inhibited by several adriamycin derivatives [19]. Experimental data similar to those reported for cytochrome *c* oxidase in fig. 1 suggest that the inhibition is mediated by interaction of the antibiotics with cardiolipin which is also essential for the activity of complex I–III [10]. Another possibility for the mechanism of inhibition is that the formation of the adriamycin-cardiolipin complex induces clustering of cardiolipin molecules into a separate lateral phase within the membrane. On the other hand, inhibition resulting from complex I–III being surrounded by adriamycin-cardiolipin complexes in the mitochondrial membrane cannot be ruled out. This mechanism is supported by the results obtained in preliminary experiments which indicate that, in the presence of water-soluble quinone (coenzyme Q_1 or duroquinone), inhibition of complex I–III by adriamycin is undetectable even at 10^{-3} mol/l (data not shown) [19]. These results suggest that inactivation could arise from the inaccessibility of complex I or III to the lipid-soluble quinone CoQ_{10} embedded in the membrane. However it should be borne in mind that CoQ_1 is a water-soluble quinone [20] capable of interacting with enzymatic sites by using a non-lipid external pathway. In order to gain more

insight into this process of inhibition, the influence of adriamycin derivatives on complex I and III should be studied separately using CoQ_{10}. However, due to its extremely low solubility in water, spectrophotometric experiments represent a difficult task to perform.

2.2. *Adriamycin-induced free radical formation (complex I–III)*

When adriamycin is added to intact mitochondria, the activity of complex I–III is inhibited, presumably for the same reasons as complex IV, through adriamycin-cardiolipin complex formation [19]. However, if the mitochondria are damaged (through sonication, for instance, or by long incubation times), the adriamycin-cardiolipin complex becomes capable of transferring electrons from NADH to cytochrome *c* in ubiquinone-depleted mitochondria with reversible reduction of the anthraquinone moiety of the adriamycin molecule [21]. Increased activity of complex I and III results from the formation of this adriamycin-cardiolipin complex in isolated heart submitochondrial particles and NADH-dehydrogenase-containing proteoliposomes [21]. The interactions of adriamycin with complex I in 'intact' or 'non-intact' mitochondrial membranes are presumably different. Indeed, it is known that quinones may interact at different sites within complex I [22] and that sonication can modify the degree of accessibility to adriamycin of the various sites. The manner in which one type of interaction is converted to the other in vivo is unknown, but studies carried out on adriamycin treated mice reveal the effects of electron transfer through adriamycin [23,24], indicating that this shift actually occurs. The consequences of electron transfer through the anthraquinone part of the molecule will be now briefly described.

Transfer of electrons through adriamycin results in enhanced chemical reactivity of adriamycin which binds covalently to cardiolipin. Infrared spectra of the adriamycin-cardiolipin complex after the electron-transfer reaction in complex I–III containing liposomes reveal a few, new absorption bands, the intensity of which increases as a function of incubation time in the presence of the electron donor NADH [25]. After a few hours of incubation in the presence of NADH, the membrane fluidity is considerably diminished and the capacity of adriamycin to transfer electrons is abolished [26]. The decrease in membrane fluidity is consistent with the occurrence of lipid peroxidation. In adriamycin-treated mice, the drug induces inactivation of complex I–III closely related to an increase in mitochondrial membrane viscosity and to lipid peroxidation [23]. In beef heart mitochondria and NADH-cytochrome *c* reductase containing proteoliposomes, transfer of electrons through adriamycin results in various membrane alterations. In both systems, the membrane fluidity, as measured on the basis of fluorescence depolarisation undergoes a drastic decrease of diphenylhexatriene and mitochondrial enzyme activity vanishes [21,26]. Lipid peroxidation takes place simultaneously [24]. The relevance of these effects observed on mitochondria or reconstituted complex I–III to in vivo systems is supported by the fact that mitochondria isolated from adriamycin-treated mice also display reduced enzyme activity, enhanced lipid peroxidation and decreased membrane fluidity [23].

Details of the process giving rise to the effects described above have been elucidated to some extent via studies on beef heart mitochondria and reconstituted complex I–III: one-electron reduction of adriamycin by NADH dehydrogenase has been demonstrated through the formation of an adriamycin free radical observed in ESR measurements ($g = 2.004$) [27]. For the 5-iminodaunorubicin (5-IDAU) derivative in which the aromatic moiety of the molecule is stabilized by the replacement of one C = O of the quinone by a C = NH group, free radial formation was not detected even at a concentration as high as 0.3 mM [34]. The appearance in the reaction medium of superoxide, hydrogen peroxide, and hydroxyl radicals suggests that the drug free radical is able to reduce molecular oxygen, producing O_2^- which undergoes dismutation to yield H_2O_2 [27]. These effects are observed with neither *N*-acetyladriamycin [21], which does not bind cardiolipin, nor 5-iminodaunorubicin [26].

The observation that adriamycin can yield free radical species in cardiac mitochondria is of cru-

cial importance. Since it has been reported that heart sarcosomes are only slightly active [29] or not at all [30] in generating radicals, mitochondria would remain the main subcellular organelles responsible for free radical formation.

3. Molecular characterization of the adriamycin-cardiolipin complex: Experimental approach

Duarte-Karim et al. [31] showed for the first time that acidic phospholipids, and more specifically cardiolipin, redistribute adriamycin into the lipophilic phase of the two-phase solvent system of Folch. The association constant of adriamycin and related anthracycline glycoside drugs with cardiolipin was determined on cardiolipin-containing monolayers spread at the air water interface using a surface-potential procedure [8]. Surface-potential data show clearly that the interaction has an essential electrostatic component. Indeed, *N*-acetyladriamycin (uncharged) does not interact with cardiolipin or other acidic phospholipids, while adriamycin (positively charged) does not interact with neutral phospholipids.

Since the fluorescence spectrum of the anthracycline drugs characterizes the dielectric constant of the medium surrounding the dye, penetration of the anthracycline moiety of the drugs into the hydrocarbon chain region of the phospholipid bilayer can be investigated by fluorescence titration of the drug using small unilamellar cardiolipin liposomes. Two different kinds of behavior can be distinguished [11]. In the first type (class I), we include drugs which display the highest association constants for cardiolipin and which are not deeply buried in the lipid bilayers. The latter result is in agreement with the quenching of adriamycin fluorescence by iodide, which shows that the bound drug is only partially embedded in the liposomal membrane [32]. Moreover, drugs of class I react specifically with cardiolipin and not with neutral lipids. Class II includes drugs with associate more weakly with cardiolipin and penetrate without specificity into the lipid bilayer. Derivatives such as daunomycin and adriamycin-14-octanoate were assigned to class II. They are more effective than adriamycin at decreasing the liposomal phase transition temperature [33]. It should be pointed out that neither the affinity for cardiolipin nor the depth of penetration into the hydrophobic part of the bilayer is related to or anticipated from octanol/water partition coefficients evaluated elsewhere [34]. In contrast, a good correlation between the drug-cardiolipin association constant and cardiotoxicity has been reported [8]. The specificity of adriamycin towards cardiolipin as compared with other negatively charged phospholipids is apparent from the association values of the constants with various phospholipids determined by adsorption of tritiated adriamycin on lipid monolayers using a surface radioactivity counter [8]: $K_a = 1.6 \times 10^6$ M^{-1} for cardiolipin, 1.8×10^4 M^{-1} for phosphatidylserine and phosphatidic acid, and zero for neutral DPPC. Since the association constant has a value of 1.6×10^6 M^{-1} for both the adriamycin-cardiolipin and adriamycin-DNA complexes, cardiolipin could clearly be a competitive target for adriamycin.

The difference in the affinity of adriamycin for cardiolipin and for other negatively charged phospholipids can be quantitatively explained by the stacking of neighbouring anthraquinone planes as revealed by specific changes in its visible absorption spectrum. The difference between the free energy of the association for adriamycin-cardiolipin ($\Delta G_{CL} = -RT \ln 1.6 \times 10^6 = -35.1$ kJ/mol) and adriamycin-PS ($\Delta G_{PS} = -RT \ln 1.8 \times 10^4 = -23.0$ kJ/mol) amounts to 12.1 kJ/mol [8]. Determination of the adriamycin self-association constant in aqueous solution indicates that the stacking of the chromophore gives rise to an additional free energy of pair formation of -11.7 kJ/mol, almost exactly sufficient to explain the difference in the association constants [8]. Binding of adriamycin to cardiolipin should therefore result in a complex including two stacked adriamycin molecules electrostatically bound on the two anionic phosphate groups of the cardiolipin. The reasons for the difference in behaviour between cardiolipin on the one hand and PS or PA on the other remain unclear. According to the above values for the free enthalpy of interaction, one can hypothesize that the conformation of cardiolipin allows two stacked molecules of adriamycin to interact electrostatically with the two negatively

charged lipid phosphates. A continuous array of stacked adriamycin molecules simultaneously allowing the maximum electrostatic interaction with cardiolipin could be built up if the average area occupied by an adriamycin dimer were similar to that occupied by a cardiolipin molecule. Computer modelling of the complex indicates that this indeed is the case. For PS and PA, the average distance between the phosphate groups could render the electrostatic binding of two stacked adriamycin molecules impossible. Using attenuated total reflection spectroscopy, Goormaghtigh et al. [35] recently demonstrated that, in the adriamycin-cardiolipin complex, the structures of both adriamycin and cardiolipin were modified as compared to the pure substances. Dichroism values indicate that the long axis of the adriamycin aromatic core is oriented at 39° C with respect to the normal of the bilayer plane. The partial disappearance of the characteristic bands of NH_3^+ is indicative of the involvement of the positively charged amino group of adriamycin in the formation of the complex. No preferential organization was observed for adriamycin alone. Since most of our data suggest [11] that adriamycin is not inserted into the lipid acyl chains, we believe that the cyclic moiety of the adriamycin molecule dips into the aqueous phase rather than inserting between the lipid acyl chains.

It has been recently been reported [36] that the accessibility of the dihydroxyanthraquinone part of the adriamycin molecule towards a soluble form of NADH dehydrogenase is reduced by 70–80% in the presence of small unilamellar vesicles (SUV) comprising egg phosphatidylcholine-cardiolipin (PC-cardiolipin SUV) (adriamycin cardiolipin molar ratio 2 : 1), suggesting the presence of two sites for the dihydroxyanthraquinone part of the adriamycin molecule: one buried in the bilayer and the other in the aqueous phase. Even though the possibility of a low-affinity type interaction between adriamycin and the lipid bilayer cannot be ruled out, it should be emphasized that, for instance, the binding of adriamycin onto phosphatidylcholine SUV reaches saturation when only about four molecules of adriamycin are bound per vesicle, composed of about 3000 phospholipid molecules [37,38].

4. Molecular structure of the adriamycin-cardiolipin complex: Theoretical approach

Over the preceding 15 years, we have been developing and refining a conformational analysis technique designed to take into account the particular characteristics of membrane molecules. This method compares favorably with experimental data when describing the conformation of phospholipids below and above the phase transition, and of sterols, drugs, and peptides inserted into a membrane. A review of this technique including a description of the force fields used to compute the conformational energy has been published [39]. The main features of the method are as follows: (1) the presence of the interface is taken into account in the potential functions by using a function describing the variation in dielectric constant at the interface; (2) the conformation of the molecules is determined at the interface (simulated by a dielectric constant gradient) for each molecule alone; (3) the molecules are then assembled until the interaction energy reaches a minimum. During this process, their own conformation is not allowed to change. Details of the assembly of the membrane components in and with monolayers have been reviewed [39].

In the present study, the saturated cardiolipin molecule has been selected for the conformational analysis. This simplification is not expected to modify the structure of the complex obtained. It should be noted that, despite the fact that a saturated cardiolipin molecule is dealt with in the conformational analysis, the calculated mean area occupied by a single cardiolipin molecule is identical to that obtained experimentally (1.2 nm^2) [8]. Lipid, antimitotic and lipid-antimitotic monolayers were assembled as described previously [39,40]. The values calculated for the interaction energy (E^{tot}) (table 1) are the sum of the cardiolipin-antimitotic interaction energy ($E^{cl\text{-}an}$) and of that for antimitotic-antimitotic interaction ($E^{an\text{-}an}$ ($E^{tot} = E^{cl\text{-}an} + E^{an\text{-}an}$). Significantly, those antimitotics which strongly inhibit the enzymatic activity of complex I–III yield high values for E^{tot} (acridine orange, 5-iminodaunorubicin, adriamycin and rubidazone) whereas low values are found in the cases of ethidium bromide, *N*-

Table 1

Values of interaction energies

Values of interaction energies (in kJ/mol): $E^{an\text{-}an}$, between antimitotic-antimitotic molecules; $E^{cl\text{-}an}$, between antimitotic-cardiolipin molecules; $E^{tot} = E^{cl\text{-}an} + E^{an\text{-}an}$, total interaction energy

	$E^{cl\text{-}an}$	$E^{an\text{-}an}$	E^{tot}
Acridine orange	−21.3	−13.8	−35.1
5-Iminodaunorubicin	−17.6	−15.9	−33.5
Adriamycin	−17.6	−16.3	−33.9
Rubidazone	−9.2	−14.6	−23.8
Steffimycin	−1.3	−10.5	−11.8
N-Acetyladriamycin	−2.1	−13.8	−15.9
Ethidium bromide	−18.8	−0.5	−19.3

acetyladriamycin and steffimycin. In the case of adriamycin, the conformational analysis shows that plane-plane interactions between the aromatic moiety of the antibiotic molecules as well as the cardiolipin-antimitotic interaction considerably stabilize the complex with cardiolipin. The cardiolipin molecules must be stacked in a cluster in order to maintain long-range adriamycin plane-plane interaction. Fig. 3 illustrates the structure of the adriamycin-cardiolipin complex. During the assembly of adriamycin with cardiolipin, the antimitotic molecules were allowed to penetrate into the cardiolipin monolayer with either the anthraquinone or sugar moiety pointing towards the hydrocarbon chain region of the monolayer. In these assemblies, cardiolipin-cardiolipin interactions are partially disrupted and their total energy is much greater, than that for the structure presented in fig. 3 (the probability of all these assemblies was calculated to be less than 1%). Further details on the electrostatic and Van der Waals energies of interaction in the adriamycin-cardiolipin complex are listed in table 2. The plane-plane interactions are rendered impossible for ethidium bromide because of its three-dimensional structure and the complex obtained with cardiolipin (fig. 4) exists only as a monomer in the membrane. In this case, steric repulsion occurs when two complexed monomers are in close proximity and results in a greater separation between cardiolipin molecules. The intervening space between the two complexed cardiolipin molecules, which is artificially filled by a homogeneous medium with a dielectric constant of value equal to 3, for the sake of this computation, will be occupied by other phospholipid molecules in real membranes. The three-dimensional structure of ethidium bromide calculated here is in good agreement with that determined in the X-ray investigation of Tsai et al. [41,42]. The conformational analysis of the adriamycin-cardiolipin complex yields results in good agreement with the following experimental observations.

(1) Adriamycin does not penetrate into the hydrocarbon chain region of the cardiolipin monolayer [11].

(2) The bilayer structure of cardiolipin is unaffected by the presence of adriamycin, as shown from its ^{31}P-NMR spectra [18].

(3) The orientation of the anthraquinone long axis with respect to the cardiolipin monolayer plane (36°) determined through the conformational analysis is in good agreement with that of 39°, established via polarized attenuated total reflection infrared spectroscopy [35].

(4) Infrared measurements (unpublished results) provide evidence that the cardiolipin and phosphatidic acid acyl chain orientations remain unmodified by adriamycin after complexation which is in support of the limited degree of participation in formation of the complex and consistent with no penetration by adriamycin molecules into the hydrophobic region of the membrane.

It is worth noting that the conformational method remains crude and does not take into account possible structural changes resulting from intermolecular interactions. It has recently been suggested that, in fact, two types of binding can take place between adriamycin and cardiolipin [43]. One type is described herein and corresponds to a high adriamycin-cardiolipin molar ratio (2 : 1).

Table 2

Contribution of electrostatic and Van der Waals energies (kJ/mol) to stability of the adriamycin-cardiolipin complex

	Electrostatic	Van Der Waals
Antimitotic-antimitotic	+4.3	−20.6
Antimitotic-cardiolipin	−15.5	−2.1
Sum	−11.2	−22.7

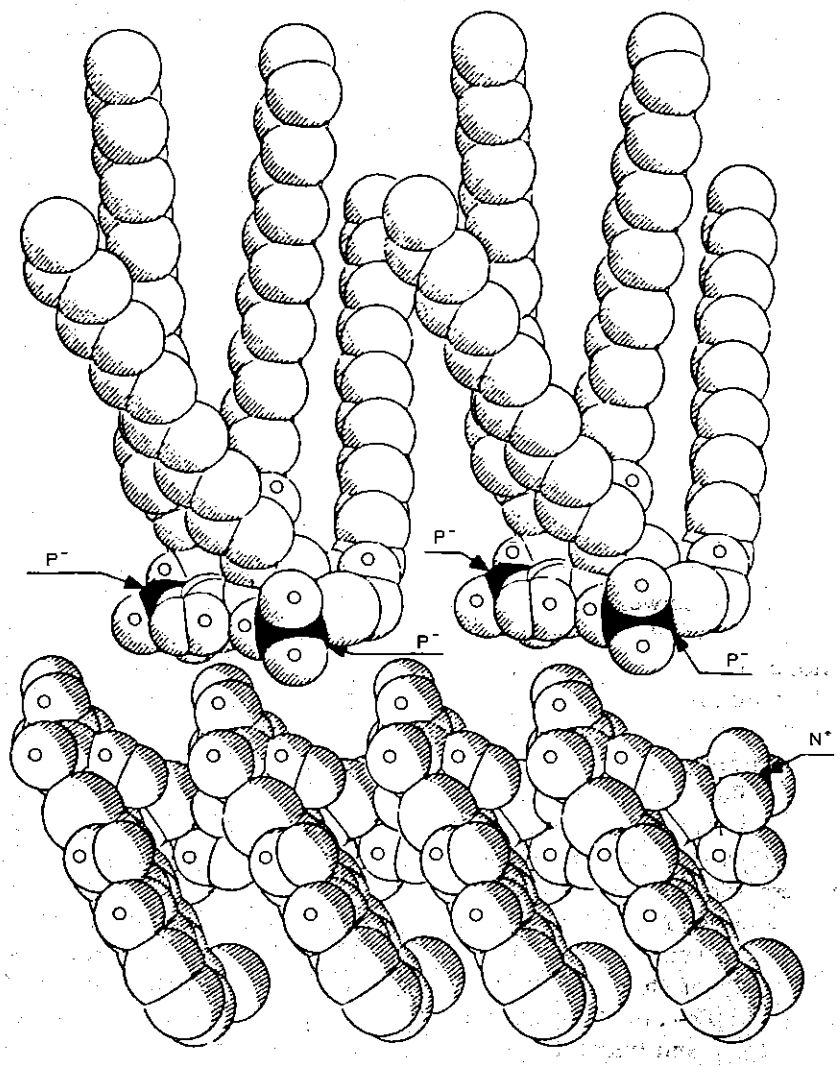

Fig. 3. Computer depiction of two cardiolipin molecules assembled with four adriamycin molecules. Arrows indicate positions of the lipid phosphate groups (P^-) and adriamycin amino groups (N^+). The plane-plane interactions between adriamycin molecules stabilize the formation of cardiolipin clusters responsible for the cardiotoxicity of adriamycin.

The other involves the fixation of adriamycin to cardiolipin through interaction of the amino group with the phosphate moiety, but with the anthraquinone ring embedded in the bilayer; it corresponds to a lower adriamycin-cardiolipin molar ratio. Nevertheless, one should bear in mind that the conformational analysis has been performed for an adriamycin-cardiolipin molar ratio corresponding to the experimental conditions for the first type of complex. The possibility exists that, for lower adriamycin concentrations, the type of organization proposed by Fiallo and Garnier-Suillerot [44] is observed; conformational analysis of this complex is currently in progress.

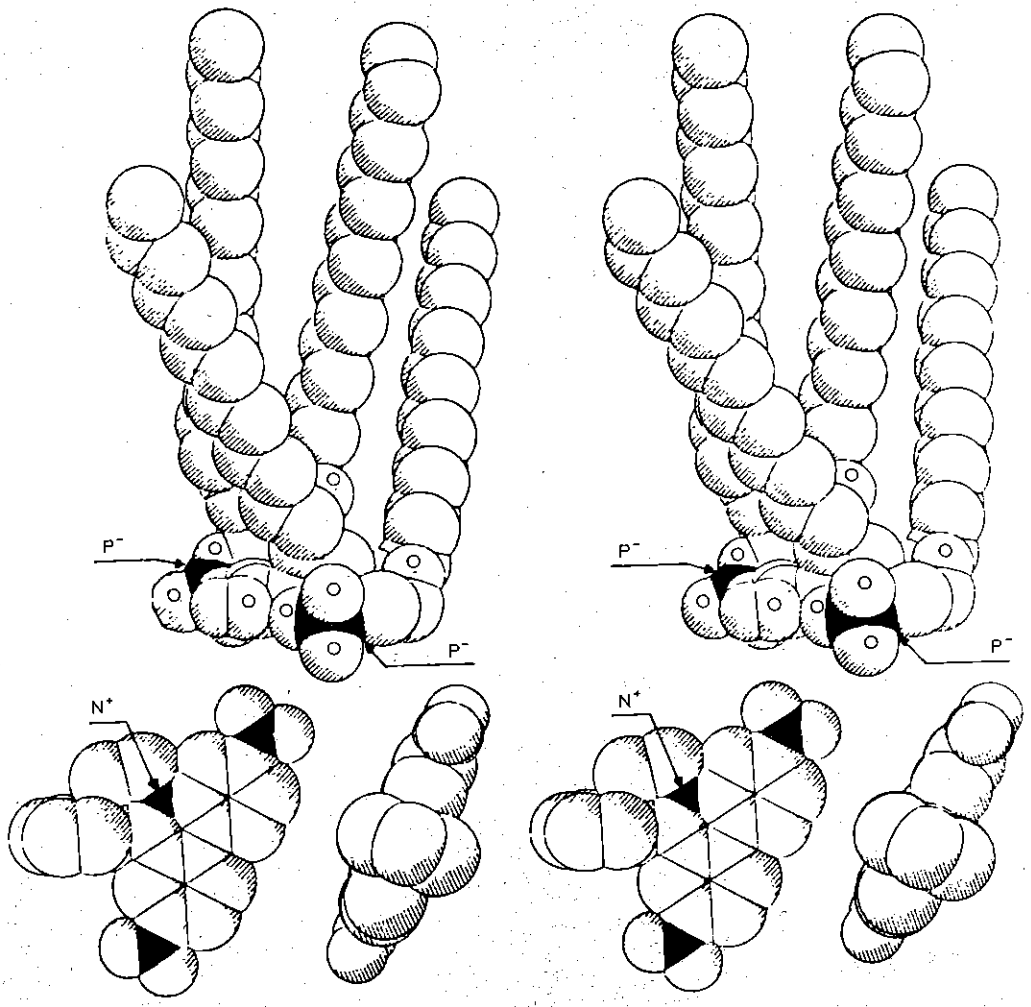

Fig. 4. Computer picture of two cardiolipin molecules assembled with four ethidium bromide molecules. P^- and N^+ have the same meanings as in fig. 3. The plane-plane interactions are rendered impossible by the drug structure and the complex obtained exists as a monomer in the membrane.

5. Perspectives

From the pharmaceutical point of view, this work provides new avenues of approach to the rational design of improved pharmacological agents. Indeed, adriamycin plays a prominent role in the treatment of leukemias and solid tumors in man [45–47] but the total dose that may be given is limited by its cardiotoxicity. Since Goormaghtigh et al. [8,11,48] have suggested that cardiolipin may be the main target responsible for this cardiotoxicity, it is tempting to design new structures which do not induce this lipid clustering or which are unable to generate free radicals while maintaining their affinity for DNA, the adriamycin nuclear target presumably responsible for its antimitotic activity. Without affecting the positively charged amino sugar of adriamycin which also stabilizes the interaction with DNA, it appears to be possible to reduce its clustering capacity and cardiotoxicity by modifying regions that are not required for adriamycin binding to DNA. The study of ethidium bromide-cardiolipin complex formation provides us with an example of

molecule with a relatively high affinity for cardiolipin [49] but without clustering effect, as demonstrated experimentally and theoretically [19]. It suggests that the introduction of new chemical groups into the adriamycin molecule which, by steric repulsion could prevent the clustering of the anthraquinone cycles, would decrease the affinity for cardiolipin. The conformational analysis which has been successful in describing the adriamycin-cardiolipin complex might be used to select less cardiotoxic antimitotics on a rational basis before undertaking any chemical synthesis.

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