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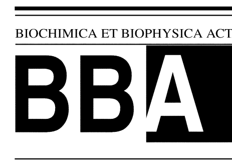

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Comparison of the interaction of doxorubicin, daunorubicin, idarubicin and idarubicinol with large unilamellar vesicles Circular dichroism study

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Abstract

Doxorubicin, daunorubicin and other anthracycline antibiotics constitute one of the most important groups of drugs used today in cancer chemotherapy. The details of the drug interactions with membranes are of particular importance in the understanding of their kinetics of passive diffusion through the membrane which is itself basic in the context of multidrug resistance (MDR) of cancer cells. Anthracyclines are amphiphilic molecules possessing dihydroxyanthraquinone ring system which is neutral under the physiological conditions. Their lipophilicity depends on the substituents. The amino sugar moiety bears the positive electrostatic charge localised at the protonated amino nitrogen. The four anthracyclines used in this study doxorubicin, daunorubicin, idarubicin and idarubicinol (an idarubicin metabolite readily formed inside the cells) have the same amino sugar moiety, daunosamine, with pK_a of 8.4. Thus, all drugs studied will exhibit very similar electrostatic interactions with membranes, while the major differences in overall drug-membrane behaviour will result from their hydrophobic features. Circular dichroism (CD) spectroscopy was used to understand more precisely the conformational aspects of the drug–membrane systems. Large unilamellar vesicles (LUV) consisting of phosphatidylcholine, phosphatidic acid (PA) and cholesterol, were used. The anthracycline–LUV interactions depend on the molar ratio of phospholipids per drug. At low molar ratios drug:PA, these interactions depend also on the anthracycline lipophilicity. Thus, both doxorubicin and daunorubicin bind to membranes as monomers and their CD signal in the visible is positive. However, doxorubicin with its very low lipophilicity binds to the LUV through electrostatic interactions, with the dihydroxyanthraquinone moiety being in the aqueous phase, while daunorubicin, which is more lipophilic is unable to bind only through electrostatic interactions and actually the hydrophobic interactions are the only detected. The highly hydrophobic idarubicin, forms within the bilayer a rather complex entity involving 2–3 molecules of idarubicin associated in the right-handed conformation, one cholesterol molecule and also molecule(s) of phosphatidic acid, as this special oligomeric species is not detected in the absence of negatively-charged phospholipids. Idarubicinol differs from idarubicin with CH(13)–OH instead of C(13)=O and its interactions with LUV are distinctly different. Its CD signal in the visible becomes negative and no self associations of the molecule within the bilayer could be detected. The variation of the sign of the Cotton effect (positive to negative) may derive from the changes in the C(6a)–C(7)–O(7)–C(1′) dihedral angle. It is noteworthy that C(13)–OH group, which strongly favours formation of the dimeric species in aqueous solutions when compared to idarubicin prevent association

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inside the LUV bilayer. At high ratios of phospholipids per drug all of them are embedded within the bilayer as monomer.
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Keywords: Doxorubicin; Daunorubicin; Idarubicin; Anthracycline; Liposome; Circular dichroism

1. Introduction

Doxorubicin, daunorubicin and other anthracycline antibiotics constitute one of the most important group of drugs used today in cancer chemotherapy [1]. Despite 20 years of widespread clinical usage, the biological activities of the anthracyclines remain to be fully elucidated and, to this end, characterisation of their membrane interactions is of importance. In this context, the interactions of anthracycline anti cancer drugs like doxorubicin or daunorubicin with membranes have been intensively studied with use of various techniques including spectroscopic methods [2–18]. The anthracycline chromophore with its rich set of the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions sensitive to the ring interactions with various molecules is very attractive object especially for absorption, CD and fluorescence spectroscopies.

The details of the drug interactions with membranes are of particular importance in understanding the kinetics of the passive diffusion of drug through the membrane which is itself basic in the context of multidrug resistance (MDR) of the cancer cells [14,19–21]. Previous studies have shown that the degree of resistance, expressed by the resistant factor of MDR cells towards antitumour drugs, decreases when the kinetics of uptake increases and thus, in the most of cases, when the lipophilicity of the molecule increases [20–23]. The role of anionic phospholipids in the interaction of moderately lipophilic doxorubicin with membrane has been widely studied [2–10], while the interactions of highly lipophilic anthracyclines such as idarubicin with biological membranes are much less explored.

Anthracyclines are amphiphilic molecules possessing dihydroxyanthraquinone ring system which is neutral under the physiological conditions. Due to the presence of different substituents, the lipophilicity of the molecules can vary within a large scale. The amino sugar moiety bound at C(7) site bears the positive electrostatic charge localised at protonated amino nitrogen. Four anthracycline drugs used in this study including idarubicinol, an idarubicin metabolite readily formed inside the cells and having antitumour activity comparable to that of idarubicin [24], have the same amino sugar moiety, daunosamine, with pK_a of 8.4. This latter value is critical for the concentration of the neutral form of the drug, which is able to enter inside the cell through the passive diffusion mechanism. Thus, all drugs studied will exhibit very similar electrostatic interactions with membranes, while the major differences in overall drug–membrane behaviour will result from their hydrophobic features.

Recently, using the observation that the anthracycline fluorescence increases when drug becomes embedded within the bilayer, we have determined the partition coefficients and the binding constants of several anthracycline derivatives to large unilamellar vesicles [11]. The results have shown that, at high phospholipid to drug molar ratio, anthracyclines were embedded within the polar head of the bilayer and that the efficiency of their incorporation in the bilayers depended neither on the negative charge on the membrane nor on the positive charge on the drug. Both, the positively charged drugs as well as the neutral ones have the same ability to be embedded within the membrane bilayer. However, the percentage of each form present should depend on the electrostatic parameters. In that respect fluorescence, or more precisely microspectrofluorescence, was a powerful technique to follow the drug behaviour in the membrane. It did not, however, provide any specific information about the drug structural conformational changes during its interactions with the bilayers.

To understand more precisely the conformational aspects of the drug–membrane interactions, we have applied circular dichroism spectroscopy and the results obtained are discussed in this paper. To compare these results with those obtained earlier with use of fluorescence techniques, the same composition of large unilamellar vesicles consisting of phosphatidylcholine, phosphatidic acid and cholesterol, was used.

2. Materials and methods

2.1. Drugs and chemicals

Doxorubicin (DOX), idarubicin (IDA), idarubicinol (IDOL) were kindly provided by Pharmacia and Upjohn laboratory and daunorubicin (DNR) by Roger Bellon laboratory. Stock solutions were prepared in water just before the experiment. Concentrations were determined by diluting stock solutions, in water to approximately 10^{-5} M and using $\varepsilon_{480} = 11{,}500\,M^{-1}\,cm^{-1}$. Egg phosphatidylcholine (PC), egg phosphatidic acid (PA) and cholesterol (CHOL) were from Sigma. The experiments were performed at 20 ± 2°C in 0.05 M Hepes buffer at pH 7.2 in the presence of 0.1 M KCl. All other reagents were of the highest quality available, and deionized double-distilled water was used throughout the experiments.

2.2. Absorption and CD spectroscopy

Circular dichroism (CD) measurements were performed using a Jobin Yvon Mark V dichrograph equipped with a thermostated cell holder. Samples were placed in quartz cuvette, 0.2 cm pathlength, and incubated at 20°C for 10 min prior to data acquisition. Typically 3 scans were collected and 2–4 replicates were made. Absorption spectra were recorded on a Cary 219 spectrophotometer.

2.3. Preparation of liposomes

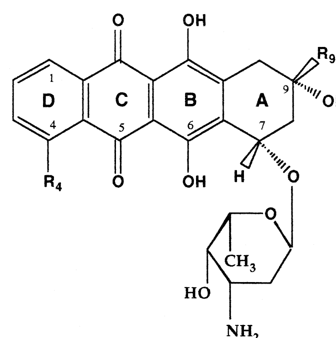
Large unilamellar vesicles (LUV) were prepared on the day of an experiment by the method of Barchfeld and Deamer [25]. Egg phosphatidylcholine, phosphatidic acid, and cholesterol were combined at PC/PA/CHOL molar ratios of 95:5:0, 75:5:20, 55:5:40, 80:20:0, 75:20:5, 70:20:10, 65:20:15, 60:20:20, 55:20:25, 50:20:30, 45:20:35, and 40:20:40. Organic solvents were removed by evaporation under nitrogen gas and then vacuum. A film containing 45 μmol of phospholipid was obtained. The lipid was redissolved with 20 ml diethyl ether and 5 ml buffer was added. The suspension was sonicated for 2 min at 0°C to produce a homogeneous dispersion, after which the diethyl ether was removed by rotatory evaporation under reduced pressure for about 30 min according to the method described by Szoka et al. [26]. The vesicle suspension was extruded through polycarbonate filters with 1, 0.4 and 0.2 μm pores, successively. Mean diameters of LUV were determined with a nanosizer apparatus (Coulter N4MD). Each sample was measured three times to obtain an average value with a standard deviation less than 10%. The mean diameter was 180 nm. The method of Marshall Stewart [27] was used to determine the concentration of phospholipid.

2.4. Hydrophobicity of the anthracycline derivatives

An estimation of the hydrophobicity of a compound is given by $\log P$, where P is the partition coefficient in an n-octanol/water system. In a first approximation, the $\log P$ value of a compound can be estimated by adding the f_i values of its fragments [28]. We have determined the variation of $\log P$ of tested compounds using daunorubicin as a reference compounds and equation

$$\log P = \log P_0 + \Sigma f_i,$$

where P_0 is the partition coefficient for daunorubicin in n-octanol/water and f_i is the n-octanol/water fragmental constant of the fragments of a compound that differentiate it from daunorubicin [29]. The values are reported in Fig. 1.



Anthracycline	R4	R9	relative lipophilicity Σf_i
Daunorubicin (DNR)	OCH3	C(=O)CH3	0
Doxorubicin (DOX)	OCH3	C(=O)CH2OH	-1.67
Idarubicin (IDA)	H	C(=O)CH3	+1.05
Idarubicinol (IDOL)	H	CH(OH)CH3	+1.48

Fig. 1. Structures and relative hydrophobicity values of the anthracyclines used. Variation of hydrophobicity evaluated as sum of fragmental values f_i, referred to daunorubicin.

3. Results

The structures of the four anthracycline derivatives used in the study are shown in Fig. 1.

3.1. Self-association of anthracyclines in aqueous solution

In aqueous solution, anthracyclines may undergo the self-association process depending on their concentrations. It is already well documented that at high concentrations drug may be present as a dimer species, whereas low concentrations favour the formation of monomeric species. The CD signal observed depends strongly on the association state of the drug and can be used as a very efficient tool to indicate whether the molecules are associated or not [30]. The CD spectrum of anthracycline in the associated state is very characteristic and exhibits a signal of the couplet type in the visible region centered at 480 nm with a positive band at $\sim$460 nm ($\Delta\varepsilon \sim +2$) and a negative one at $\sim$540 nm ($\Delta\varepsilon \sim -1$) (Fig. 2). This characteristic feature was used to determine the association state of anthracycline in the presence of LUV.

The association constant corresponding to the dimer formation depends strongly on the presence of the hydroxyl group in position C(13) as well as on that of the methoxy group at C(4). The comparison of the CD spectra of doxorubicin and daunorubicin (2×10^{-3} M) with those of idarubicin, that lacks methoxy group at C(4), shows that while doxorubicin and daunorubicin exist as dimeric species, idarubicin is present mainly as a monomeric species. However, the presence of an hydroxyl group in position C(13) causes that idarubicinol exists mainly as a dimer. Since the CD spectra of the dimeric species exhibit well defined characteristic negative band at $\sim$ 540 nm, not seen in the spectra of the monomeric anthracyclines (Fig. 2), it is possible to use its $\Delta\varepsilon$ plotted as a function of the drug concentrations to estimate the dimerization constant. The calculated values (data not shown) for the association constants were found to be $1.1 \pm 0.2 \times 10^4$, $8.6 \pm 1.6 \times 10^3$ and $2.0 \pm 0.5 \times 10^4\ \mathrm{M}^{-1}$ for doxorubicin, daunorubicin and idarubicinol, respectively. In the case of idarubicin, even at very high drug concentrations such as $3 \times 10^{-3}\ \mathrm{M}^{-1}$, only about 20% of the molecules are present as dimeric species and we have just estimated that the order of magnitude of the constant was $100\ \mathrm{M}^{-1}$.

The anthracycline concentration used throughout the following study was $5 \times 10^{-4}\ \mathrm{M}^{-1}$. Experiments were also performed at lower concentrations (100 and 50 μM) and the data did not depend on the anthracycline concentration.

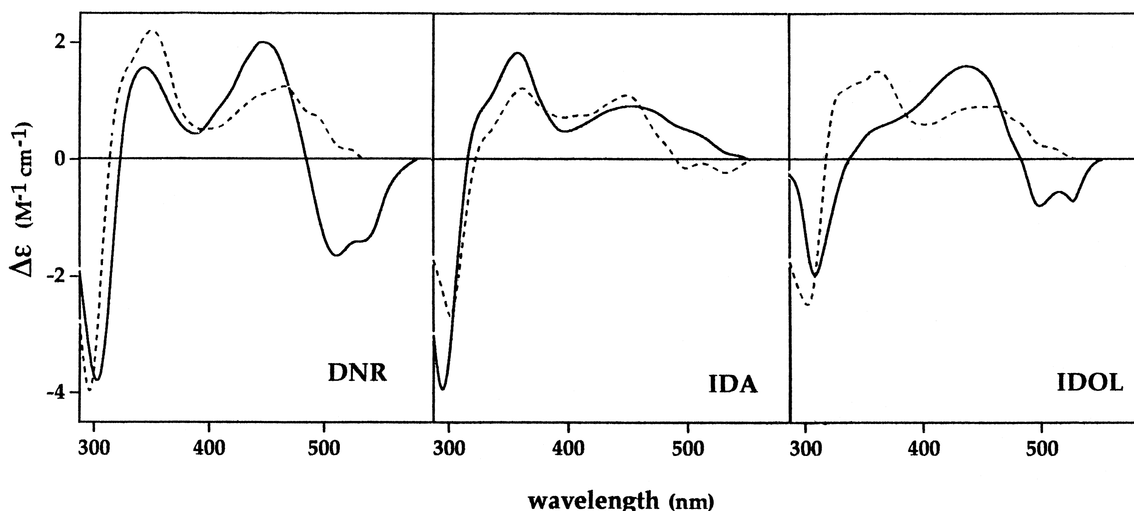


Fig. 2. Circular dichroism spectra of daunorubicin (DNR), idarubicin (IDA) and idarubicinol (IDOL) at 10^{-3} M (————) and 10^{-5} M (- - -).

3.2. Interaction of doxorubicin and daunorubicin with LUV

The interaction of doxorubicin with liposome has already been studied by different groups and the specific role of the anionic phospholipids is well described [2–10]. The CD spectra obtained in this work indicate that when LUV membranes contain high amount of anionic phospholipids (20%), two step binding of drug molecule can be easily detected. At low molar ratio PA:DOX, the molecules are bound through electrostatic interactions involving the negative charge of the phosphatidic acid and the positive charge of the amino sugar [7]. The CD spectrum of the couplet type characteristic for the free dimeric doxorubicin in aqueous phase disappears and is replaced by a positive band of relatively high intensity (band I) (Fig. 3(a)). The presence of an isodichroic point at 450 nm indicates that an equilibrium between mainly two species, free and bound drug, is present. In this interaction pattern, the dihydroxyanthraquinone moiety lies outside the bilayer in the bulk of the aqueous medium. When the molar ratio PA:DOX increases, the intensity of this positive band decreases and its simultaneous shift to longer wavelengths is observed (band II). This spectrum change is due to the embedding of the dihydroxyanthraquinone moiety inside the bilayer and a prevalence of the hydrophobic interactions as compared to the electrostatic ones. Both, band I and band II are characteristic of monomeric species. The isodichroic point between band I and II (500 nm) indicates that monomeric species I, with dominantly electrostatic drug–LUV interactions, and monomeric species II, with dominantly hydrophobic interactions, are in equilibrium in the system studied.

When similar CD measurements are performed in the presence of low amount of anionic phospholipid (5%), no band I characteristic for the electrostatic interactions is observed. The obtained spectrum changes continuously when LUV is added. An isodichroic point (480 nm) from the spectrum of the dimer in aqueous solution to that of the monomer embedded in the bilayer (band II) is observed. This species yielding band II will hereafter be called monomer II or ''membrane monomer''. The CD spectra of doxorubicin, characteristic for the molecules in the dimeric form in aqueous solution and bound to the membrane either as monomer I or monomer II, are shown in Fig. 3(b). The presence of cholesterol in the bilayer does not modified the features of these two spectra.

Our experiments have shown that the best conditions to observe the transformations from drug in the

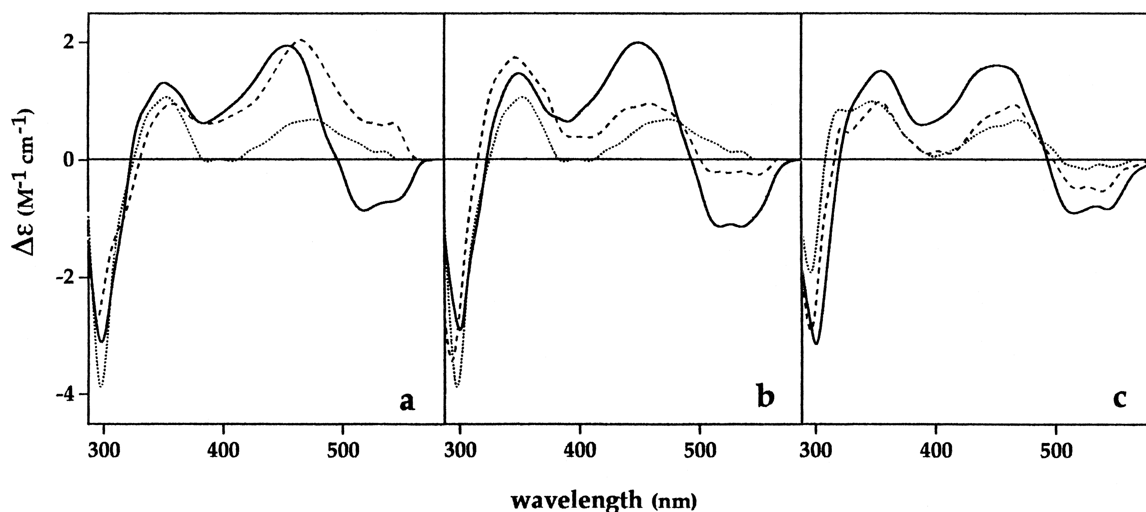


Fig. 3. Circular dichroism spectra of doxorubicin (DOX) and daunorubicin (DNR) in the absence of LUV (————), in the presence of LUV at PA:Anthracycline = 1:2 (- - -), in the presence of LUV at PC:anthracycline = 15 (· · ·). [Anthracycline] = 5×10^{-4} M, DOX plus LUV containing 20% PA (a); DOX plus LUV containing 5% PA (b); DNR plus LUV containing either 5 or 20% PA (c).

dimeric species to monomer I and then to monomer II are in the presence of LUV containing 20% PA following the series:

aqueous dimer (no LUV)

$\rightarrow$ monomer I (PA:DOX = 1:2)

$\rightarrow$ monomer II (PL:DOX $>$ 10).

Using LUV containing none or very low amount of PA the equilibrium is:

aqueous dimer (no LUV)

$\rightarrow$ monomer II (PL:DOX $>$ 10).

Under the physiological pH conditions, daunorubicin bears exactly the same charge as doxorubicin and its electrostatic interaction with membrane should be the same. However, dihydroxyanthraquinone moiety is more lipophilic and, therefore, the hydrophobic interactions are expected to be stronger than those observed for doxorubicin. The CD spectra show that indeed under the same conditions as those used for doxorubicin, daunorubicin does not exhibit band I when LUV are added, (Fig. 3(c)), and the only species observed is monomer II, with drug molecule embedded within the polar head of the membrane. Thus, the CD spectra of daunorubicin, measured with LUV for different PA:DNR molar ratios exhibiting isodichroïc point at 480 nm (Fig. 3(c)), indicate that daunorubicin molecule moves directly from the aqueous phase to the embedded site within the polar head region and the interactions characteristic for monomer I are of minor, if any, importance for the DNR–LUV system. Also in this case, the presence of cholesterol has no influence on the CD spectral pattern of monomer II.

3.3. Interactions of idarubicin with LUV

Idarubicin molecule is distinctly more lipophilic than both doxorubicin and daunorubicin. However, the presence of the same sugar moiety should yield the same electrostatic interactions as those expected for doxorubicin and daunorubicin. On the other hand, at the concentration used in this study, 5×10^{-4} M, only 5% of molecules of idarubicin are in the dimeric form and the CD spectrum is characteristic for a monomeric species.

The addition of LUV, that contains none or low amount of phosphatidic acid, gave rise to small modification of the CD spectrum indicating that the drug molecule gets embedded inside the bilayer yielding the membrane monomer. This spectral modifications did not depend on the presence of cholesterol (Fig. 4).

However, the addition of LUV that contains 20% of PA with different amounts of cholesterol yields very typical CD spectra exhibiting a signal of the couplet type with a negative band at 435 nm and a positive one at 480 nm (Fig. 4). This CD signal characteristic for dimeric species, that further we will call type I′, can be characterised by the A-value equal to $\Delta\varepsilon_1$-$\Delta\varepsilon_2$, where $\Delta\varepsilon_1$ and $\Delta\varepsilon_2$ are the Cotton effects at the longer and shorter wavelengths, respectively. Fig. 5 shows the plot of the A-values as a function of the molar ratio PL:IDA for different amounts of cholesterol present in the LUV membrane. The data show that the A-value reached a maximum at PL:IDA molar ratio of 1 for all amounts of cholesterol used. The A-value obtained at PL:IDA equal to 1 as a function of the CHOL:IDA molar ratio increases up to a ratio of 0.3–0.4. At PL:IDA molar ratios higher than $\sim$4 the CD spectrum characteristic of the membrane monomer (band II) reappears.

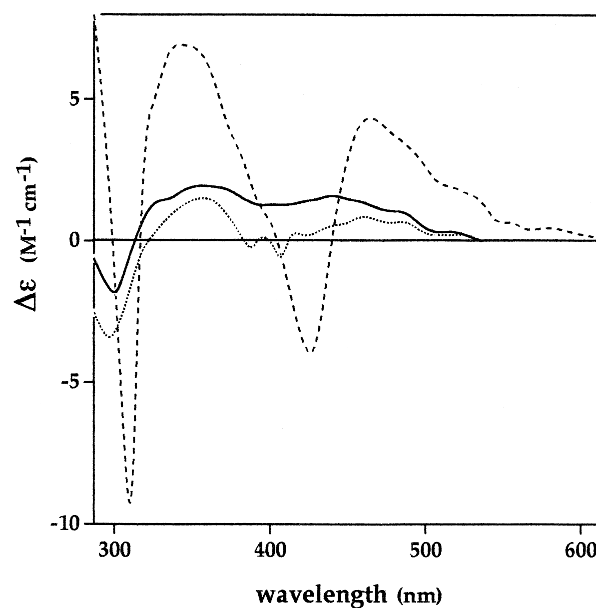


Fig. 4. Circular dichroism spectra of idarubicin in the absence of LUV (————), in the presence of LUV containing PC 40%, PA 20%, CHOL 40% at PL:IDA = 1:1 (- - -); 5:1 (· · ·).

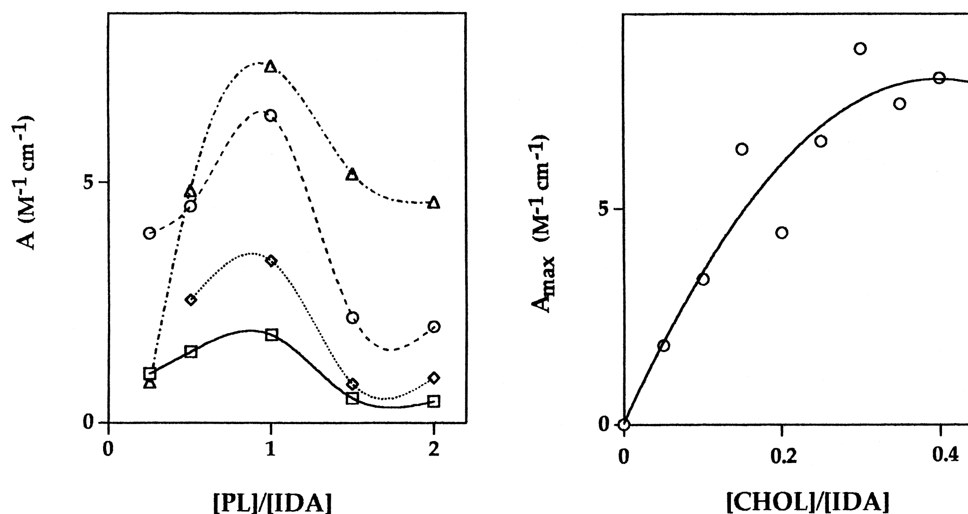


Fig. 5. Circular dichroism of idarubicin (IDA) in the presence of LUV containing PA 20% and various amount of CHOL and PC. Left: $A = \Delta\varepsilon_1 - \Delta\varepsilon_2$ has been plotted as a function of the ratio PL:IDA for different amounts of cholesterol (□, 5%; ◇, 10%; ○, 15%; △, 35%). Right: the $A = \Delta\varepsilon_1 - \Delta\varepsilon_2$ value obtained at PL:IDA = 1 has been plotted as a function of the ratio CHOL:IDA.

3.4. Interaction of idarubicinol with LUV

At the concentration used here LUV-free idarubicinol is almost 100% in the dimeric form. Also idarubicinol has the same charge on the amino sugar as the other three compounds and, thus, the electrostatic interactions are expected to be very similar to those of the other drugs studied here. However, idarubicinol is the most lipophilic molecule among all four derivatives discussed here.

The addition of LUV that contained 5% of phosphatidic acid and no cholesterol gives rise to the appearance of the CD spectrum characteristic of a monomer embedded in the bilayer (band II, Fig. 6). However, the addition of LUV containing 20% PA and 20–30% CHOL with the PA:IDOL molar ratio of 0.5, results in a CD spectrum exhibiting a negative band in the visible region. For PL:IDOL molar ratios higher than 4 one can observe the CD spectrum with band II characteristic for membrane monomer species.

3.5. Absorption spectra characterisation of the anthracycline–LUV interactions

The absorption spectra of anthracycline depend strongly on the solvent used. The characteristic vibrational fine structure of the absorption band observed in the visible region becomes better resolved when dielectric constant of the solvent decreases. As an example, Fig. 7 shows the absorption spectrum of idarubicin dissolved in water and DMF, respectively. Similar spectral modifications are observed for other three compounds (data not shown).

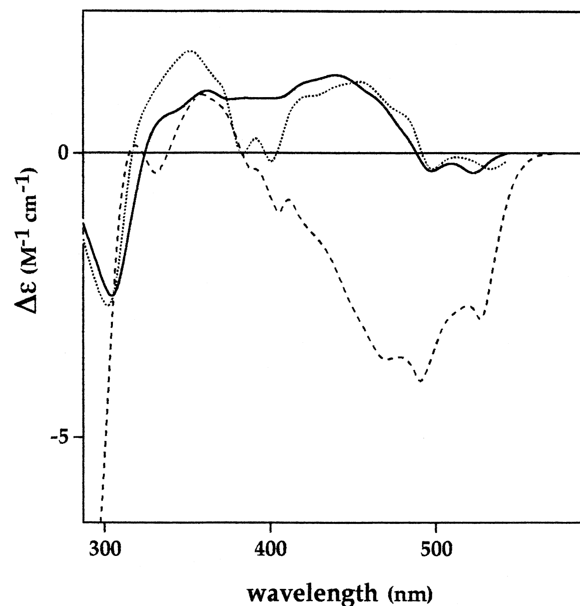


Fig. 6. Circular dichroism of idarubicinol (IDOL) in the absence of LUV (————), in the presence of LUV containing PC 40%, PA 20%, CHOL 4O% at PL:IDOL = 1:2 (- - -) or 5:1 (· · ·).

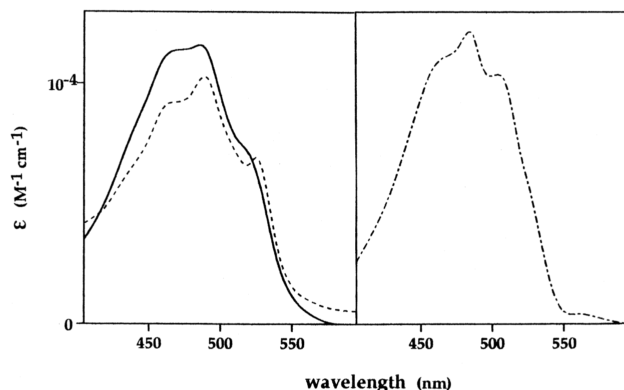


Fig. 7. Absorption spectra of idarubicin in water (————), in DMF (– · –), in the presence of LUV containing PC 40%, PA 20%, CHOL 40% at PL:IDA = 1:1 (- - -).

The absorption spectra of doxorubicin and daunorubicin bound to the LUV membrane do not show well resolved vibrational structure, while the absorption spectra of idarubicin and idarubicinol interacting with LUV membranes show vibrational fine structure comparable to that observed for free drugs dissolved in DMF (Fig. 7). This clearly indicate that both idarubicin and idarubicinol are embedded deeper in the bilayer than the two other drugs doxorubicin and daunorubicin.

4. Discussion

The impressive affinity of doxorubicin for negatively-charged phospholipids has been the subject of numerous investigations [2–10]. The ability of doxorubicin to participate in such charge–charge interactions at pH 7.4 is due to its protonated amino group, with a $pK_a = 8.4$ at 25°C [31]. Modification of this pK_a value can strongly modulate the drug interactions with charged membranes and, using doxorubicin analogues with low pK_a values, it was shown that they do not bind selectively to the negatively charged phospholipids [14].

Our study concerns four anthracycline derivatives which have the same amino sugar and, therefore, the same electrostatic interaction with LUV, but different lipophilicity and thus, different ability for embedding within the bilayer. The conformational modifications that drugs undergo through interactions with LUV allow to evaluate the effect of lipophilicity on these drug–membrane interactions.

Our previous studies have shown ultimately that at high molar ratio of PL:drug, the same type of binding is observed for all four derivatives: they are embedded as monomeric species inside the bilayer [11]. In addition, doxorubicin and daunorubicin are embedded within the membrane at the level of the polar head, i.e. in a medium with an electric permittivity comparable to that of methanol [11,32]. However, the method used did not permit to localise both, idarubicin and idarubicinol, in the membrane bilayer. Nevertheless, the appearance of the well resolved vibrational fine structure in the absorption spectrum allows to conclude that these two lipophilic compounds are embedded deeper within the bilayer than doxorubicin and daunorubicin.

Burke et al. [15,16] have examined the effect of valerate substitution at C(14) on the anthracycline associations with electroneutral dimyristoyl phosphatidylcholine (DMPC) bilayers, as well as with negatively-charged bilayers composed of homogeneous dimyristoyl phosphatidylglycerol or with a binary mixture of DMPC and cardiolipin. C(14)-valerate substitution was found to eliminate the selective binding of several anthracyclines to negatively-charged phospholipids, thereby suggesting that a simple synthetic modification might prevent the accumulation of positively-charged anthracyclines in membranes abundant in negatively-charged lipid. However, another interpretation can be given and this effect would not be due to the specific substitution at C(14) but to the fact that the compound thus obtained is much more lipophilic than doxorubicin. Actually, in the present paper, we show that the selective binding of anthracycline to negatively-charged membrane is also eliminated with compound such as idarubicin where there is no C(14) modification but an increase of the lipophilicity due to the lack of the methoxy group at C(4).

We have recently discussed the influence of the dissymmetry elements of the anthracycline structures on their CD spectra [33]. The molecular shape of the anthracyclines is determined by (i) the conformation of the sugar ring, (ii) the conformation of the A ring and (iii) the orientation of the sugar with respect to the aglycone moiety [34]. Because of the chromophore–sugar distance, the conformation of the sugar ring should not affect the CD spectra. Concerning the cyclohexene ring (A), it has been reported

that two half-chair conformations are favoured. The conformation called α is typical of most anthracyclines and is favoured by the hydrogen bond between C(9)–OH and C(7)–O and the longer distance between phenolic C(6)–OH and C(7)–O, which decreases the repulsive interaction between the two corresponding dipoles [35]. The conformation called β is observed for 6-deoxyanthracycline derivatives (no C(6)–C(7) repulsive interaction) [36] and for 8-(*S*)-fluoroanthracycline derivative (configuration at C(8)) [37]. The CD spectra of idarubicin (α conformation) and 8-(*S*)-fluoroidarubicin (β conformation) are the same and CD spectroscopy cannot allow the distinction between the two conformations. It follows that the geometry of the glycosidic linkage would have a great impact on the CD signal amplitude at 480 nm. The geometry of the glycosidic linkage can be defined by the torsional angle C(6a)–C(7)–O(7)–C(1′). In monomeric anthracycline derivatives, in aqueous solution, the mean plane of the sugar is perpendicular to the dihydroxyanthraquinone plane. In the case of the ''membrane monomer'' (species II) which is obtained for all anthracyclines at high molar ratio of phospholipid per drug, the CD signal is positive, its intensity is lower than that observed for the monomer in aqueous solution and comparable to that of the corresponding aglycone. This strongly suggests that in the ''membrane monomer'' the value of the C(6a)–C(7)–O(7)–C(1′) torsional angle has changed when compared to the free drug molecule.

At low molar ratio of PA:drug, different anthracycline behaviours are detected depending on their lipophilicities. Thus, doxorubicin, with its very low lipophilicity, binds to the LUV through electrostatic interactions with the dihydroxyanthraquinone moiety being in the aqueous phase whereas daunorubicin, which is more lipophilic, is unable to bind only through electrostatic interactions and the hydrophobic interactions already play the major role.

The case of idarubicin deserves special comments. As it was mentioned above the anthracycline derivatives when dissolved at high concentration in aqueous solutions dimerize and, according to the CD signal of the couplet type at 480 nm with a negative *A*-value, the molecules are associated in the left-handed conformation. Both doxorubicin and daunorubicin bind to membranes as monomers, while the very hydrophobic idarubicin, which is more deeply embedded within the bilayer than the two others, forms a rather complex entity involving 2–3 molecules of idarubicin associated in the right-handed conformation, one cholesterol molecule and also molecule(s) of phosphatidic acid, as this special oligomeric species is not detected in the absence of negatively-charged phospholipids.

Idarubicinol differs from idarubicin with C(13)–OH instead of C(13)=O and its interactions with LUV are distinctly different. It does not form any associations within the bilayer membrane, as it is attested by the presence of only one band in the visible. In addition, this band is negative and it should be noticed that in the case of daunorubicin which, at low molar ratio of phospholipid per drug, is also embedded within the bilayer as a monomer, this signal is positive. The variation of the sign of the Cotton effect may derive from the changes in the C(6a)–C(7)–O(7)–C(1′) torsional angle and it can be suggested that this is due to the proximity of the sugar moiety to the C(5)=O and C(6)–OH functions to mask the positive charge. It is noteworthy that C(13)–OH group, which strongly favours formation of the dimeric species in aqueous solutions when compared to idarubicin prevent association inside LUV bilayer.

In conclusion, our data show that, whereas the interactions of doxorubicin and daunorubicin with membrane are not strongly influenced by the presence of cholesterol, the interactions of the more lipophilic idarubicin and idarubicinol depend on the presence of cholesterol, concomitantly with that of negatively charged phospholipid. In a biological context, it means that idarubicin and idarubicinol can recognize cholesterol-rich membrane, whereas doxorubicin and daunorubicin cannot. The present findings are of particular interest from a drug-design standpoint, revealing that lipophilicity may be more important for the recognition of cholesterol-rich phospolipids membrane than it has previously been considered [38].

Acknowledgements

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