PARTITIONING OF EU(III) COORDINATION COMPLEXES INTO LIPID BILAYER

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The present study is focused on development of liposomal delivery systems for two newly synthesized drugs with high anticancer activity – Eu(III) coordination complexes, referred to as V3 and V4. These pharmacological preparations belong to a new class of antineoplastic drugs, whose high cytotoxic potential has been demonstrated very recently. Liposomes composed of phosphatidylcholine (PC) were chosen as effective nanocarriers due to their undisputable advantages such as enhanced drug solubility, reduced toxicity, improved stability, etc. The results of preformulation studies are presented, describing UV absorption spectroscopy-based evaluation of the degree of drug loading into liposomes which strongly determines the therapeutic and toxic effects of the pharmacological compounds. Drug association with the lipid bilayer was followed by the absorbance increase with maximum position being independent on lipid concentration. Zwitterionic nature of PC and relatively high hydrophobicity of the pharmaceuticals allowed us to conclude that drug-lipid binding is governed preferentially by hydrophobic interactions. Higher efficiency of encapsulation into the lipid bilayers was found for V3 ($K_p = 1.4 \times 10^5$) compared to V4 ($K_p = 6.7 \times 10^3$). This effect was interpreted in terms of V3 influence on bilayer molecular organization giving rise to facilitated partitioning of this drug into the vesicle interior.

KEY WORDS: partition coefficient, Eu(III) coordination complexes, drug-lipid interactions

Within the last decade biomedical research has been revolutionized by high-throughput development of a diversity of multifunctional nanostructures including nanoparticles, nanotubes, quantum dots, micelles, liposomes, dendrimers and many other nanoassemblies providing unique opportunities for diagnosis, treatment and prevention of a number of severe diseases. Among these liposomes offer a vast number of advantages including, particularly, biocompatibility, complete biodegradability, non-toxicity, ability to carry both hydrophilic and lipophilic payloads and protect them from chemical degradation and transformation, increased therapeutic index of drug, flexibility in coupling with targeting and imaging ligands, improved pharmacokinetic and pharmacodynamic profiles compared to free drugs, reduced side effects, etc. [1,2] Liposome-incorporated pharmaceuticals are protected from the inactivating effect of external conditions, yet do not cause undesirable side reactions. Liposomes provide a unique opportunity to deliver pharmaceuticals into cells or even inside individual cellular compartments. Size, charge and surface properties of liposomes can be easily changed simply by adding new ingredients to the lipid mixture before liposome preparation or by variation of preparation techniques. Another important feature is that lipid vesicles can entrap both hydrophilic and hydrophobic pharmaceutical agents [3,4]. While their lipidic bilayer help solubilizing hydrophobic compounds, their internal aqueous center provides a way of encapsulating hydrophilic drugs. Particular attention is currently given to the development of liposomal formulations of new classes of antineoplastic drugs with alternative mode of cytotoxic action and nonoverlapping mechanisms of drug resistance. One of such classes is represented by lanthanide coordination complexes whose high cytotoxic potential has been demonstrated very recently [5]. The therapeutic and toxic effects of particular drug are strongly determined by the degree or efficiency of its loading into the liposomes. An important parameter for biological activity of vesicle-entrapped pharmacological agents is their partition coefficients into the lipid phase. Lipophilicity of chemical compounds is very often described as partition coefficient in the octanol/water system. But the octanol–water partition model does not adequately reflect the drug behavior in the living systems. The alternative use of liposomes has become more widespread. Also, liposomes contain the main ingredients found in all biological membranes [3]. The present study is directed towards the evaluation of lipid-binding ability of two newly synthesized coordination complexes of Eu(III), referred to as V3 and V4. Since V3 and V4 are hydrophobic compounds, zwitterionic lipid phosphatidylcholine (PC) was chosen for preparation of lipid vesicles.

MATERIALS AND METHODS

Egg yolk phosphatidylcholine was purchased from Biolek (Kharkov, Ukraine). Lipid vesicles composed of PC were formed using extrusion technique [6]. The thin lipid film was obtained by evaporation of lipids' ethanol

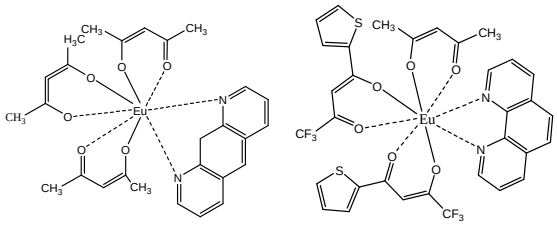

Fig. 1. Structure of lanthanide complexes.

solutions and then hydrated with 1.2 ml of 5 mM Na-phosphate buffer (pH 7.4). Lipid suspension was extruded through a 100 nm pore size polycarbonate filter. Phospholipid concentration was determined according to the procedure of Bartlett [7]. Absorption measurements were conducted using SF-46 spectrophotometer against solvent blanks. V3 and V4 (Fig. 1) were synthesized as described previously [5].

RESULTS AND DISCUSSION

As seen in Fig. 1, V3 and V4 are asymmetric Eu(III) coordination complexes with diverse O-containing chelate ligands which, apparently, serve at least two main functions – bind tightly Eu(III), providing the rigidity of the whole-molecule structure, and shield lanthanide ion from quenching and destabilizing effects of water. V3 and V4 also contain organic chromophores which are responsible for absorbing the excitation light and transferring the energy to the lanthanide. Fig. 2 represents typical absorption spectra of Eu complexes under study in the absence and presence of lipid vesicles. Despite the fact that these compounds suffer from low extinction coefficients, they are characterized by broad absorbance spectrum in the range 240-320 nm with the peak at 266 nm. Association of V3 and V4 with model membranes is followed by the marked increase in drug absorbance while absorption maximum was found to be virtually independent on lipid concentration. The enhancement of drug absorption in liposomal suspension can be rationalized in terms the two main factors: (i) drug transfer to the lipid environment of reduced polarity, (ii) immobilization of lanthanide complexes within the lipid matrix. Zwitterionic nature of PC molecules and relatively high hydrophobicity of V3 and V4 allowed us to assume that drug-lipid association is controlled mainly by hydrophobic interactions. Particular attention should be given to the question of drug location within the liposomes. As can be judged from the drugs' structures, CH_3-groups (in the case of V3) and CF_3- and sulfur-containing groups (in the case of V4) reside, apparently, in the polar region of bilayer or at polar/nonpolar interface acting as anchors while hydrophobic ring systems interact presumably with acyl tails of lipid molecules.

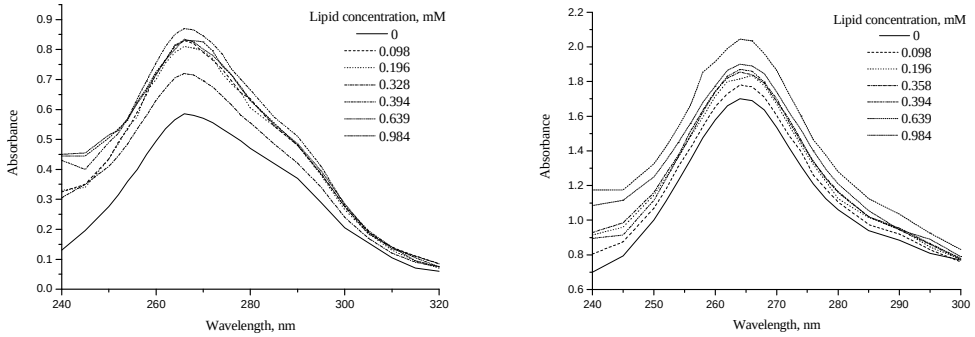

Fig.2. V3 (A) and V4 (B) absorption spectra in PC model membranes. Drug concentration was 40 μM.

Drug partition coefficient into the lipid phase is defined as [8]:

$$K_p = \frac{(C_m/C_t)/[lipid]}{(C_w/C_t)/[water]} \quad (1)$$

where C_t is the drug molar concentration, the subscripts m and w stand for lipid and aqueous media, $[lipid]$ and $[water]$ represent lipid and water molar concentrations, respectively. The relationship between K_p and absorbance increase upon formation of drug-lipid complexes can be written as:

$$\Delta A = \frac{K_p \varepsilon C_t [lipid]}{[water] + K_p [lipid]} \quad (2)$$

where $\varepsilon = \varepsilon_m - \varepsilon_w$ [9], ε_m and ε_w are the drug extinction coefficients in the lipid bilayer and water, respectively. To derive the partition coefficients for the lanthanide complexes, the experimental dependencies presented in Fig. 3 were approximated by Eq. (2).

The K_p values for V3 and V4 were found to be *ca.* 1.4×10^5 and 6.7×10^3, respectively, suggesting higher affinity of V3 for the neutral bilayers. It is tempting to suppose that different lipophilicity of the examined drugs originates from the peculiarities in their structures. It should be noted that chemical nature of the entrapped drug strongly affects its partitioning into the membrane [10]. The more pronounced lipid-associating ability of V3 can, in principle, be attributed to higher percentage of nonpolar CH_3-groups.

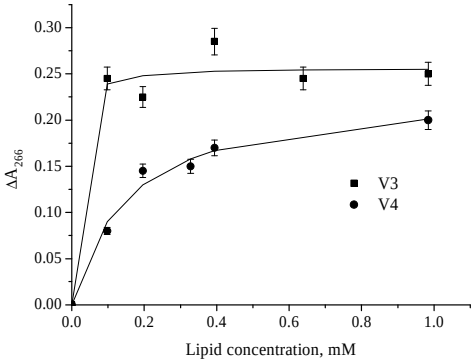

Fig. 3. The isotherms of V3 and V4 binding to PC model membranes. Solid lines represent theoretical curves providing the best fit to the experimental data. Drug concentration was 40 μM for both compounds.

However, it cannot be excluded that V3 and V4 are capable of affecting the molecular architecture of PC bilayer. Particularly, specific interactions of lanthanide molecular groups with the lipid headgroups may modify the hydrogen bonding within the bilayer and destabilize it. This, in turn, would weaken the lipid packing, increase the free volume and facilitate the drug partitioning into the vesicles. These considerations point to scenario in which lanthanide molecules perturb *per se* lipid bilayer promoting thereby self-partitioning in the liposome interior as it was observed by Custodio *et al.* for 4-hydroxytamoxifen [10]. However, to confirm the validity of the above suggestions, further studies directed towards the examination of K_p dependence on drug concentration are required.

CONCLUSIONS

Overall, the present study strongly suggests that newly synthesized anticancer drugs V3 and V4 can be efficiently entrapped by the lipid phase of PC vesicles, thereby paving the way for the development of their liposomal formulations. Determination of drug partition coefficients revealed that chemical structure of the compounds is crucial for their incorporation into lipid matrix. The observed differences between K_p values of V3 and V4 are explained by different abilities of these drugs to alter molecular organization of lipid bilayer.

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