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Langmuir monolayers and Differential Scanning Calorimetry for the study of the interactions between camptothecin drugs and biomembrane models

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ABSTRACT

CPT-11 and SN-38 are camptothecins with strong antitumor activity. Nevertheless, their severe side effects and the chemical instability of their lactone ring have questioned the usual forms for its administration and have focused the current research on the development of new suitable pharmaceutical formulations. This work presents a biophysical study of the interfacial interactions of CPT-11 and SN-38 with membrane mimetic models by using monolayer techniques and Differential Scanning Calorimetry. The aim is to get new insights for the understanding of the bilayer mechanics after drug incorporation and to optimize the design of drug delivery systems based on the formation of stable bilayer structures. Moreover, from our knowledge, the molecular interactions between camptothecins and phospholipids have not been investigated in detail, despite their importance in the context of drug action. The results show that neither CPT-11 nor SN-38 disturbs the structure of the complex liposome bilayers, despite their different solubility, that CPT-11, positively charged in its piperidine group, interacts electrostatically with DOPS, making stable the incorporation of a high percentage of CPT-11 into liposomes and that SN-38 establishes weak repulsive interactions with lipid molecules that modify the compressibility of the bilayer without affecting significantly neither the lipid collapse pressure nor the miscibility pattern of drug–lipid mixed monolayers. The suitability of a binary and a ternary lipid mixture for encapsulating SN-38 and CPT-11, respectively, has been demonstrated.

1. Introduction

Irinotecan (camptothecin CPT-11; 7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxycamptothecin) and SN-38; (irinotecan metabolite, ethyl-10-hydroxy-camptothecin) are antineoplastic agents belonging to the family of topoisomerase I inhibitors that arrest the synthesis of DNA and possess strong antitumor activity (Fig. 1) [1,2].

The sole catalytic mechanism for camptothecin action consists in the formation and stabilization of a reversible enzyme–drug–DNA ternary complex which prevents the ligation step of the nicking/sealing cycle performed by the topoisomerase enzyme. CPT-11 is converted to its metabolite SN-38, with a reported, at least, 100-fold biggest activity, by a human carboxylesterase (hCE), primarily in the liver [3], but also in tumors [4]. CPT-11 is a first-line drug approved for the treatment of a variety of human tumors, including colorectal, lung and gynecological cancers [5]. Both CPT-11 and SN-38 are currently in clinical trial in its liposomal form [6]. However, their severe side effects, such as myelosuppression and gastrointestinal disorders [7,8], impose some restrictions for camptothecin therapies and additional considerations to develop suitable pharmaceutical formulations for clinical purposes. Other drawbacks for their clinical applications are the chemical instability of the lactone ring, which opens to the inactive carboxylate form at physiological pH [9,10] and, in the case of SN-38, the great insolubility in almost all the solvents that could be used to formulate this drug. All of these considerations make evident the importance of protecting the drug from the external environment to maximize its pharmacological potential and the need of using solubilizers or membrane stabilizers [11]. Therefore, current investigations are focused on the development of new forms for camptothecin administration.

Abbreviations: CPT-11, irinotecan, 7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxycamptothecin; SN-38, irinotecan metabolite, ethyl-10-hydroxy-camptothecin; DSC, Differential Scanning Calorimetry; P, partition coefficient; DSPC, L-α-Distearoyl-phospathidylcholine; DOPS, L-α-Dioleoyl-phospathidylserine; EPC, Egg Phosphathidylcholine; CHOL, Cholesterol; MLVs, Multilamellar liposomes.

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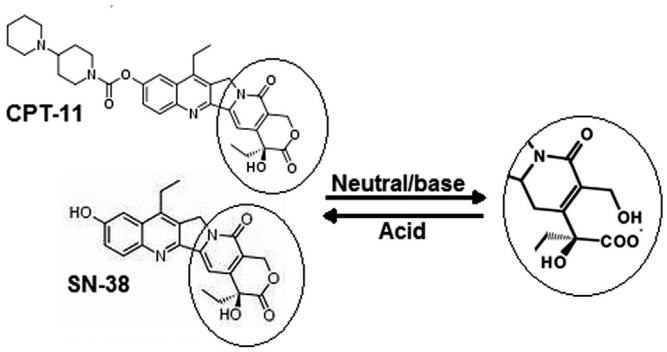

Fig. 1. Molecular structure of camptothecins. Equilibrium between their lactone and carboxylate forms.

The consideration of the pharmaceutical form of anticancer drugs and the procurement of stable formulations can overcome some of the main limitations for their use in clinical applications. Docetaxel, for example, is available in a formulation that contains a high concentration of Tween 80 [12] and Paclitaxel is often provided in Cremophor EL. Unfortunately, the use of both products has been associated with severe side effects related with hypersensitivity reactions [13], nephrotoxicity and neurotoxicity. In order to solve the problems derived from the use of such solubilizers or adjuvants, alternative dosage forms have been developed to improve its clinical administration. Among the potential drug delivery carriers, liposomes or lipid vesicles have endowed with interesting and useful characteristics that make them a pivotal biocompatible and biodegradable drug delivery and formulation platform [14].

Liposomes possess many interesting properties, such as the ability to entrap both hydrophilic and hydrophobic molecules without loss or alteration of their activity, which make them very suitable to create triggered release systems and to provide passive or active targeted strategies [15,16]. They may carry different surface charges, have different sizes and provide long systemic circulation times, depending on their lipid composition. Liposomes can act as sustained depots which release their cargo in a controlled form and in a specific target, giving a preferential accumulation in solid tumors [17]. Moreover, liposomal formulations can reduce the toxicity associated with free anticancer drugs in healthy tissues [18,19], which is severe in the case of CPT-11 and SN-38, and improve drug stability by protecting the compound from chemical degradation or transformation. Several lipid-based and liposomal nanomedicines have been approved in the last 20 years [20–22] and many others are undergoing clinical evaluation [23].

In the case of camptothecins, their encapsulation in liposomes would have the additional advantages of providing a suitable environment to maintain soluble the drug, either in the aqueous phase or in the lipid bilayer, and to afford protection for the lactone ring of the molecule, which is essential for its pharmacological activity, besides being an important structural requirement both for the passive diffusion of these drugs into cancer cells and for their successful interaction with the topoisomerase I enzyme [24,25]. Moreover, CTP-11 liposomalization increases its antitumor activity with an important reduction in the adverse reactions reported for this drug, being the use of carriers completely essential in the case of SN-38, because of its extreme insolubility, to make it a useful drug [26].

Liposomes can be engineered from a wide variety of lipid species, from natural or synthetic origin and can be endowed with special characteristics by adding to their formulation specific components. You can make liposomes sensitive to specific stimuli, stable as pharmaceutical products and in the biological media after administration and that can be vectorized to specific and targeted locations. To develop optimal drug formulations and efficient drug delivery systems it is essential to control the physicochemical parameters of the vehicle and, for this purpose, it is very useful to study how molecular interactions between the constituents of the carrier and the drugs can affect or modify its structure [27,28]. Moreover, the study of drug–lipid interactions can also be used to predict the pharmacokinetic properties of drugs, which are dependent on their chemical stability and, consequently, their bioavailability and efficacy.

When characterizing the interactions of drugs with membrane lipids, it is essential to consider the use of different techniques, each one with advantages or limitations regarding to their applications [29,30]. Among these, it could be outlined Differential Scanning Calorimetry (DSC) [31,32] and Langmuir monolayers [33,34].

DSC is a nonperturbative technique largely employed in pharmaceutical thermal analysis, because its ability to provide information about either the physical or energetic properties of substances. Moreover DSC is one of the more used methods to measure the enthalpy associated with physical processes [35]. As a thermoanalytical method, DSC has definite applications in nanosciences with important features for the development of nanostructured lipid carriers for drug delivery [36,37].

The Langmuir techniques, which use lipid monolayers at the air–water interface as the model for studying the two-dimensional arrangement, are very useful in the area of liposome formulation as they provide information on lipid packaging configuration and so, on liposome stability [34,38]. In addition, the knowledge of the partition coefficients (P) that can be determined in an n-octanol/aqueous medium [39] or by a reverse phase HPLC column [40], gives a good approach to predict the relative tendency of drugs to incorporate into biological membranes.

This work explores the physicochemical interactions of CPT-11 and SN-38 with pure and mixed lipid monolayers and bilayers and informs about the potential usefulness of the liposomal carriers designed for these drugs [41]. The long term stability is an essential parameter of the final formulation because it will control the sustained release of these camptothecins into the cell and because of the protection afforded to their cargos versus its biological degradation. The results will provide comprehensive insights about the possible effects of the molecular interactions of these drugs, on the liposomal formulation, either at the level of the hydrophobic domain of the lipid bilayer in which they can be inserted, or at the level of the polar region when encapsulated in their aqueous space, always in contact with the inner monolayer of the bilayer. It could also be emphasized that, from our knowledge, the molecular interactions between camptothecins and phospholipids have not been investigated in detail.

2. Experimental

2.1. Materials

L-α-Distearoyl-phospathidylcholine (DSPC), L-α-Dioleoyl-phospathidylserine (DOPS), Egg Phosphathidylcholine (EPC) and Cholesterol (CHOL) were purchased from Avanti Polar Lipids (Birmingham, AL, USA). CPT-11, purchased from Afine Chemicals Limited (Hangzhou, China), was pure with a minimal grade of 99%. SN-38 was from Tocris Bioscience (Bristol, United Kingdom). All the organic solvents (Panreac, Montcada i Reixac, Barcelona, Spain) have been distilled before use. Milli-Q water (Millipore Bedford, Massachusetts system, resistivity of 18 MΩ cm) was used. All other chemicals and solvents were of analytical grade.

2.2. Calorimetric studies

Differential scanning calorimetric (DSC) analysis was used to evaluate the thermodynamic aspects of the camptothecin/lipid interactions and was performed by using a Mettler DSC-30 device (Mettler-Toledo, Inc., Columbus, OH, USA) or a MicroCal VP-DSC (GE Healthcare LifeSciences, Uppsala, Sweden). The calorimetric systems were calibrated, in transition temperature and enthalpy changes, by