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Biophysical Interactions of 5-Fluorouracil with lipid bilayers: a molecular dynamics study

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Abstract: Liposomal drug delivery systems are increasingly being used in various fields of biology and medicine such as in non-invasive cancer chemotherapy. It has been shown that drugs can be encapsulated by liposomes while their release is controlled by membrane proteins sensitive to mechanical, optical or magnetic forces. Drug-lipid interactions can alter the physiochemical properties of lipids and/or their embedded proteins which may in turn compromise drug efficacy. Unlike drug-protein interactions, far less focus has been put on studying direct influence of certain drugs on their surrounding lipids. Here using all-atom molecular dynamics (MD) simulation, we studied the effect of encapsulated Fluorouracil 5-FU, a drug widely used in cancer therapy, on a POPE lipid bilayer system. In our system, we have also modelled a bacterial channel, MscL, which has recently been proposed for use as a nanovalve for liposomal drug delivery purposes. Our results clearly indicate that properties such as bilayer lateral pressure profile, bilayer thickness, lipid order parameter, drug permeation, and channel conformation are largely influenced by 5-FU.

Keywords: Drug Delivery Systems; Nanovalve; Liposome; Cancer; MscL

Introduction

There has been a prodigious effort and interest to make cancer drugs less invasive and more effective. One of the best methods has been put forward over the past few years is liposomal drug delivery systems[1, 2]. In this method, drugs are encapsulated inside the liposomes and are realised by different actuators such as membrane-embedded particles or proteins [3]. The caveats to this technology are the possibility of drugs interacting with the embedded protein and/or with their surrounding membrane which may ultimately reduce the drug efficiency. Although drug-protein interactions are well known, the biological ramification of drug-membrane interactions is unclear.

Recently, genetically modified bacterial mechano-sensitive channel MscL, reconstituted into the liposomes, has frequently been proposed in recent literature as a suitable nanovalve for triggered release of beneficial compounds such as drugs [4-8]. Here using all-atom MD simulations, we investigated interaction of 5-FU, a drug widely used in cancer therapy, on a model lipid bilayers containing MscL. Fluorouracil, 5-fluoro-2,4(1H,3H)-Pyrimidinedione (5-FU) is a pyrimidine analog that is an antineoplastic antimetabolite used for GI, breast, and head and neck cancers [9, 10].

In a separate but similar computational system, we also studied the effects of an anesthetic drug, trifluoroethanol (TFE). We then compared the effects of both drugs on both lipid bilayer and the embedded MscL channel.

Materials and method

All molecular dynamic simulations were performed with the NAMD 2-10 package, where CHARMM36 Force Field was employed for lipid/protein system. Visual Molecular Dynamics (VMD) and PYMOL were used for all visualizations. The 3D structure of MscL channel was generated based on the crystal structure of E. coli MscL. The resultant MscL models were embedded into a 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-ethanolamine (POPE) bilayer comprised of 222 lipid. The lipid heads and tails were in turn randomised and equilibrated for ~1 ns at 298 K, while the rest of the system was fixed. Then the protein and lipids were next solvated with a 120 × 120 × 130–Å water box. After randomization of the POPE lipid tails (1 ns), the whole system was equilibrated for 62 ns with a time step of 2 fs with no restraints. The equilibration steps have been done identically to our previous MD simulation of EcMscL [11]. Then we added 2% v/v Fluorouracil (5-FU) ($C_4H_3FN_2O_2$) molecules into one side of bilayer leaflet. The force field parameters for 5-FU is obtained from CHARMM GUI [12] to see the effect of 5-FU, we equilibrated the system for 15 ns and then performed the main run for 45 ns. We used the MEMBPLUGIN SCD Order Parameter Tool [13] to compute the S_{CD} (deuterium order parameters) of lipid tails. Also we wrote custom tcl codes for calculating the pressure profiles and thickness of lipids as well as changes in channel configuration.

Results and Discussion

The effect of 5-FU and TFE on bilayer permeation, bilayer thickness, lateral bilayer pressure profile, order parameter and channel conformational change have been examined using all-atom MD simulation (Fig.1-3). As demonstrated in Fig.1, 5-FU molecules were adsorbed more readily and reside deeper within the bilayer (below the phosphate group) compared to the TFE molecules despite being ~ 1.5 times larger in size and molecular weight. Yet like TFE, 5-FU molecules cannot completely

penetrate the hydrophobic barrier. This result suggests high adsorption of 5-FU by non-treated lipids such as POPE. Interestingly, unlike TFE, 5-FU reduces both sn-1 and sn-2 order parameters indicating that the bilayer becomes more disordered. These effects are more pronounced toward the bilayer mid-plane (Fig.2). Moreover, 5-FU greatly influences the lateral pressure profile of the bilayer (Fig.3). The area under the lateral pressure profile is almost two-fold larger once the drug is incorporated into the bilayer. This could be due to the size, shape and hydrophilicity of 5-FU. In addition, the difference between the pressure in upper and lower leaflets of the bilayer can induce a local curvature which can per se modulate the activity of the embedded channel [14,]. From our simulations, it is evident that in the absence of any external force, the effect of 5-FU on MscL conformation is more than that of TFE. Incorporation of 5-FU into the bilayer changes the MscL outer diameters by ~ 30 %.

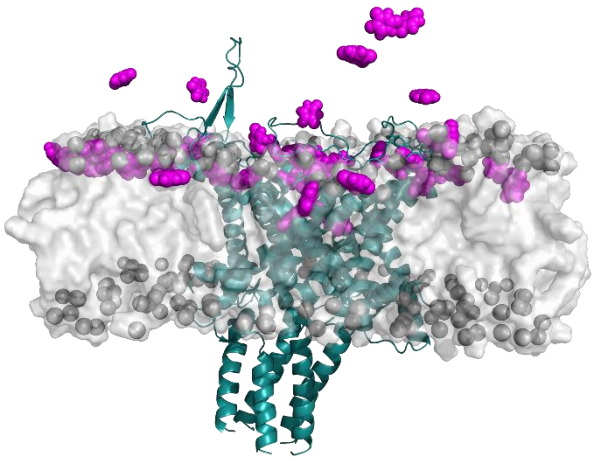

Fig. 1. Adsorption of 5-FU into a POPE lipid bilayer containing MscL obtained from MD simulation.

Conclusions

Using all-atom MD simulation, we examined the dynamic interaction of two drugs, 5-FU (used for cancer treatment) and TFE (an anesthetic) on a POPE lipid bilayer. Overall both drugs have large effects on the physicochemical properties of the bilayer such as bilayer permeation, bilayer thickness, lateral bilayer pressure profile, order parameter and channel conformational change. The effects of 5-FU were more dramatic compared to TFE. Here we have identified the main technical challenges associated with using liposomal preparations for the delivery of 5-FU for clinical practice such as in cancer chemotherapy.

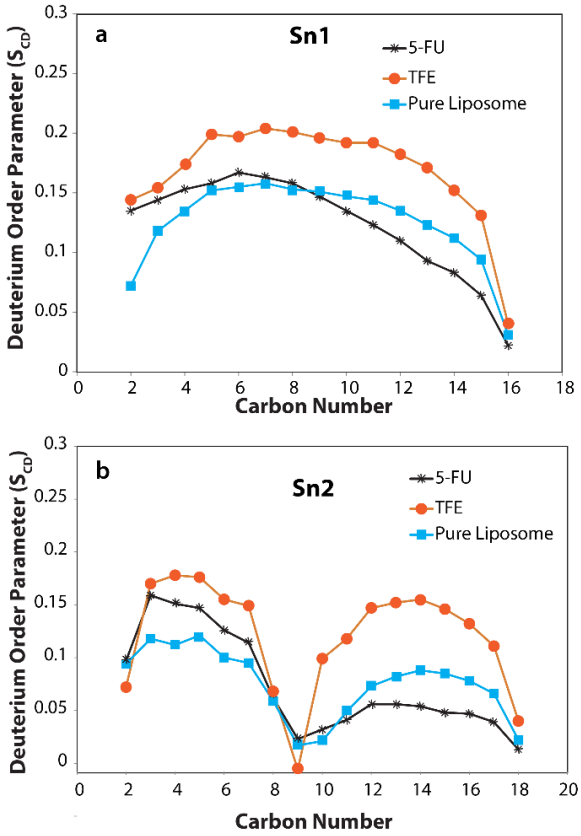

Fig.2. Effect of 5-FU on A) Sn-1 order parameter and B) Sn-2 order parameter of a POPE bilayer containing MscL.

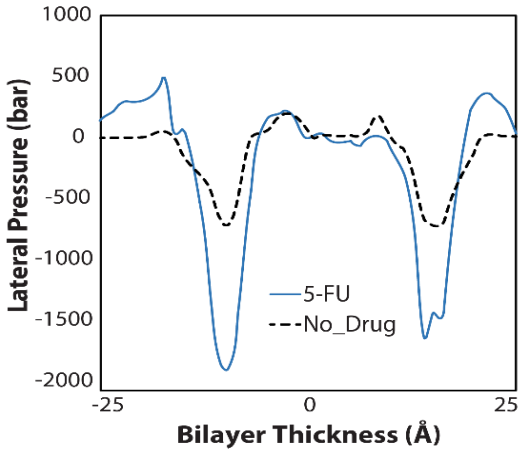

Fig.3. Effect of 5-FU on the lateral pressure profile of the bialyer containing MscL.

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