Dynamical and Structural Properties of Cytarabine in a Lipid Bilayer: A Molecular Dynamics Study

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Abstract
Cytarabine is a chemotherapy agent that has found use in the treatment of cancers of white blood cells such as acute myeloid leukemia. Molecular dynamics simulation was carried out to investigate the interaction between the anticancer drug cytarabine and a DOPC/Chol lipid bilayer. MD simulations allowed one to estimate the position, orientation, and dynamics of cytarabine molecules inside the membrane. Cytarabine molecule was found to reside in the phospholipid headgroup area in the bilayer. Additionally, we found among the functional groups of cytarabine, OH groups form maximum hydrogen bonds with both of the DOPC and Chol molecules, while N3 group forms minimum hydrogen bonds.

Keywords: Liposomal Cytarabine; Lipid bilayer; Molecular dynamics simulation; DOPC; Cholesterol.

1. Introduction
A main goal in drug design researches is to specify delivery systems that enhance drug efficacy at the intended site of action. It has been well established that liposomes improved therapeutic index for many drugs by a decrease in drug toxicity and an increase in therapeutic potency. Protection the drug from metabolism and ability to carrying both of water-soluble and lipid-soluble drugs causes liposomes are applied as a drug carrier in some of diseases, especially in cancer treatment [1]. Application of molecular dynamics (MD) simulation methods gave insight on full details of molecular interactions between liposomal membrane and drugs. Cytarabine, also known as cytosine arabinoside (C9H13N3O5) is a chemotherapy agent used mainly in the treatment of cancers of white blood cells such as acute myeloid leukemia (AML). It kills cancer cells by interfering with DNA synthesis and blocking the progression of cells from the G1-phase to the S-phase. Liposomal cytarabine, DepoCyt, is a sustained-release formulation of the cytarabine, ensuring prolonged cytotoxic drug concentrations of cytarabine in cerebrospinal fluid [2]. We modeled a bilayer membrane consisting DOPC and cholesterol molecules as the membrane for carrier of cytarabine to study drug-bilayer interaction. In this study, molecular dynamics simulations were applied to study drug-bilayer interactions in cholesterol (chol) containing membranes.
2. Computational details

2.1. Simulation systems

In this study, we modeled a membrane containing 76 DOPCs and 52 Chol molecules (40% Chol). Starting configuration of the model membrane was taken from pre-equilibrated systems. Visual Molecular Dynamics (VMD) software was used for insertion of one cytarabine molecule to build initial structure.

2.2. Simulation parameters

We used the united-atom approach (GROMOS 43A1-S3 force field) to DOPC and cholesterol molecules. The water molecules and the hydroxyl group of cholesterol were treated with full atomic details. Molecular structure and topology of cytarabine molecule were obtained using the PRODRG2 server.

2.3. Simulation conditions

All MD simulations were performed using the GROMACS package [3]. Cutoff radius of 12 Å was employed for van der Waals interactions. To treat long-rang electrostatics interactions, the particle mesh Ewald (PME) algorithm was applied. All simulation were carried out at constant temperature (323 K), which is above the main phase transition temperature for a pure DOPC bilayer (238 K), and constant pressure (1 atm), semi-isotropically. Both the temperature and pressure of the system were controlled by the Berendsen method. After energy minimization, 5 ns of equilibration with harmonic restraints on the coordinates of the cytarabine atoms were performed. Finally, production run was performed for 25 ns under an NPT ensemble. The last 20 ns of each MD run were taken for calculating averages. The atomic coordinates were saved every 4 ps for analysis.

3. Results and discussion

3.1. Location of cytarabine in the lipid bilayers

The effect of drug insertion on the structure of the lipid bilayer was examined by the electron density profile across the bilayer normal (the Z axis). Figure 1 shows the preferred location of the various parts in the system: head group, phosphate, choline, terminal methyl, tail group, cholesterol, water and drug. The electron density profiles were calculated from the last 20 ns of the NPT simulation. The reason for using only the last 20 ns of the trajectory is that cytarabine molecule, which were initially inserted in the center of the bilayer migrated to the interface between polar and hydrophobic membrane regions and remained there for the rest of the simulation time. In this case, the lowest density is located in the OH groups of cholesterol (15 e/nm$^3$) and the bilayer core (40 e/nm$^3$) and the highest density at the lipid/water interface (160 e/nm$^3$), with the bulk water phase in the middle (290 e/nm$^3$). Also, all lipid components, including head and tail groups are symmetrically distributed. As can be seen from Figure 1, functional groups (CO, NH$_2$ and OH) of the cytarabine molecule occupy the region between the tail groups of bilayer and bulk water. Density profile of the phosphate groups is partly overlapped by the profiles of choline groups. Also, further analysis such as order parameter, area per lipid, packing of atoms and radial distribution function (RDF) were done (data not shown).
3.2. Intermolecular interactions of cytarabine molecule

Table 1 shows the number of hydrogen bonds between individual functional groups of cytarabine and DOPC/Chol. By watching the trajectory of simulation, we see cytarabine orients its polar fragment towards the water/lipid interface. 85% of H-bonds with cholesterol are made via the OH and NH2 groups and 15% via the N3 and O2 groups. The number of NH2 (cytarabine)-DOPC H-bonds is 8% higher than OH (cytarabine)-DOPC ones.

<table>
<thead>
<tr>
<th>Cytarabin group</th>
<th>Chol group (OH)</th>
<th>DOPC group (PO4- or C=O)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH2</td>
<td>0.022</td>
<td>1.667</td>
</tr>
<tr>
<td>OH</td>
<td>0.234</td>
<td>1.938</td>
</tr>
<tr>
<td>N3</td>
<td>0.013</td>
<td>0</td>
</tr>
<tr>
<td>O2</td>
<td>0.03</td>
<td>0</td>
</tr>
</tbody>
</table>

3.3. Conclusions of cytarabine-bilayer MD simulation

Results of the MD simulation show cytarabine is located in that part of a bilayer, which interacts with polar head groups of DOPC and cholesterol molecules. Both of the electron density profiles and hydrogen bond analysis confirm this issue. Theses outcome are beneficial for liposomal drug design, especially, for cancer.

References