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Molecular dynamics simulation study of PEG-PCL nanoparticles for BCNU delivery to treat glioblastoma

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SUMMARY

Glioblastoma, the most lethal form of brain cancer is characterized by rapid growth and invasion and poor survival of the patients. Despite advances in standard therapy, including surgical resection followed by radiation and chemotherapy, glioblastoma remains highly resistant to treatments. Thus, new approaches to design optimized therapies are needed. Poly(ethylene glycol)-poly(ε-caprolactone) (PEG-PCL) copolymers are synthetic biomedical materials with high biocompatibility, biodegradability, long-circulating and enhanced drug accumulation at the site of action that are all suitable for long-term delivery. In this study, we carried out Molecular Dynamics simulation on PEG-PCL2 to investigate the ability of this nanoparticle as a drug carrier for BCNU. Simulation results indicated that PEG-PCL2 aggregated and formed stable nanoparticles with the average radius of gyration of 2 nm. Also, plots represented that BCNU penetrated deep into the micelles. In conclusion, observations confirmed that the PEG-PCL nanoparticles have the potentials to be used as BCNU delivery carriers.

Key words: glioblastoma, BCNU, molecular dynamics simulation, polymeric nanoparticle, drug carrier

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INTRODUCTION

Glioblastoma Multiform (GBM) is the most common primary brain cancer, also known as the most aggressive type of brain tumors. An annual incidence of 4-5 cases per 100 000 people results in an estimated 20000 newly diagnosed primary brain tumors in the United States [1]. The prognosis of GBM patients remains poor with a reported median survival period of approximately 15 months [2]. Despite decades of intensive work on multiple therapeutic targets, the current standard treatment for GBM narrows in surgical resection, followed by radiotherapy and chemotherapy [3]. The Blood-Brain Barrier (BBB) remains a major obstacle in the way of drugs reaching brain tumors. Although alkylating agent 1,3-bis(2 chloroethyl)-1-nitrosourea (BCNU, Carmustine) is claimed to be active against GBM in large prospective clinical trials [4]; however, GBM treatment is far from settled due to the inability of most molecules to cross the BBB.

Nanoparticles have been demonstrated as feasible drug delivery systems to improve the efficacy of chemotherapy against GBM [5, 6]. Nanoparticles increase the number of delivered drugs and thus shrinkage of tumors more effectively than spherical micelles. Drug encapsulation causes perfect isolation of the drug from the environment, thus leading both to a significant reduction of side effects and efficient drug release at the target site. Among the compounds which are employed in drug delivery systems, Polyethylene Glycol (PEG)-a biocompatible polymer- is widely used as the hydrophilic part of amphiphilic block copolymers in pharmaceutical applications which results in surprising advantages including prolonged blood circulation time, decreased antigenicity and immunogenicity, non-toxicity, solubility in water and significant tumor accumulation [7, 8]. Poly-E-Caprolactone (PCL) is a semi-crystalline polyester considered as a promising candidate for clinical trials due to its properties such as non-toxicity and gradual hydrolysis to monomers [9]. PEG-PCL copolymer, characterized by its high biodegradability, biocompatibility and long-circulating properties, is already approved by regulatory authorities such as U.S. Food and Drug Administration (FDA) for tissue engineering and drug delivery [10, 11].

Molecular Dynamics (MD) simulation method has emerged as a powerful technique for designing drug delivery vehicles providing a molecular view of the drug loading, vehicle stability at

various temperatures and the degree of drug-vehicle interactions. The MD tool is used in the present research to model BCNU encapsulation in MePEG17-b-PCL2 (PEG-PCL2) micelles, to analyze thermodynamic integration, to quantify drug partitioning and to finally to characterize drug dispersion.

MATERIALS AND METHODS

Gromacs

A software package (version 5.0.7) was used to perform the MD simulations. The MARTINI Force-Field (MFF) [12, 13] was utilized for coarse-graining the atoms in the simulation box, in which nearly every four atoms- excluding H- are lumped into a single bead. The simulations were performed with the time step of 20 fs. Cubic simulation cells were used for the simulations involving PEG-M-PCL chains in water. The self-assembly simulations involving the copolymers and BCNU drug were all performed in cubic simulation cells with a length of 12.21590 nm, with 60 PEG-M-PCL and 15 BCNU molecules placed at random locations and then solvated with 13454 MARTINI coarse-grained water beads. The shift function starting at 0.9 nm with a cut-off of 1.2 nm was used for the dispersion interactions. All the simulations were performed in an isothermal-isobaric (NPT) ensemble, with the temperature-controlled using a velocity-rescaling thermostat [14] and a coupling time of 1.0 ps. Similar to the atomistic simulations, the Berendsen barostat [15] with a coupling time of 4.0 ps was used for equilibration purposes, and the Parrinello-Rahman barostat [16] with a coupling time of 6.0 ps was used in the production runs. Compressibility of 5×10^{-6} bar^{-1} was used, with the reference pressure set to 1.0 bar. In both the atomistic and coarse-grained simulations, periodic boundary conditions were applied in all three directions. To visualize the molecules, Visual Molecular Dynamics (VMD) software [17] was utilized.

The previous model presented by Raman et al. [18] for MePEG-PCL was used to simulate the polymers self-assembly, in which the PCL repeat unit is modeled by two interaction centers. The ester part is modeled as a non-polar acceptor bead ('Na' MARTINI bead type), while the alkyl $(CH_2)_4$ part is modeled as an apolar 'C1' MARTINI bead type. In this model, the MePEG part parameters were taken from Lee et al. [19]. The length of MePEG17-PCL2 (PEG-PCL2) were selected to study self-assembly and drug delivery. For mapping and parametrization of the BCNU drug auto-MARTIN, I script was utilized [20].

RESULTS AND DISCUSSION

Radius of gyration

We carried out all-atom MD simulations to explore the self-assembly of BCNU and PEG-PCL2 micelle in aqueous solution for 40 ns. The radius of gyration (Rg) represents the compactness of the structure during the simulation, which relates to the aggregation size distributions. Rg was estimated based on the average distance of an atom from its Center of Mass (COM). The radius of gyration is a good indicator of the spatial conformation of the molecule, a higher value representing a higher spatial disposition. The radius of gyration gradually decreased due to the impact of hydrophobic interactions until the structure reached a stable position. The radius of gyration of micelles and BCNU is represented in Figure 1.

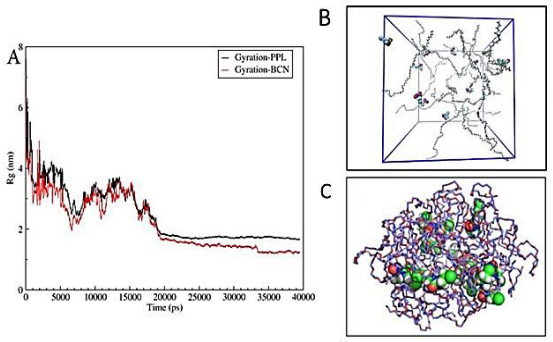

Fig. 1. Radius of gyration (Rg) fluctuation versus time for PEG-PCL2 during the self-association simulations in the presence of BCNU (1A); Simulated spontaneous aggregation shown at 0 ns (1B) and 20 ns (1C). PPL-BCN: PEG-PCL2; BCN: BCNU

Figure 1A shows a sharp decline in Rg for PEG-PCL2 and BCNU. The Rg started at around 6nm when all the micelles and drugs are randomly distributed (Figure 1B), and then it had a significant drop to approximately 3.5 nm at 3 ns, observations that affirm the PEG-PCL2 aggregated effectively and micelles were formed. After that, the figure fluctuates around 3.5 nm until 15 ns, followed by a considerable decrease, nearly reaching 2 nm at 20 ns and Rg of PEG-PCL2 remained stable towards the end of simulation, while Rg of BCNU had another gradual decline representing penetration of BCNU into the micelles (Figure 1C).

Solvent accessible surface area (SASA)

Aggregation of hydrophobic drugs in an aqueous media is driven by hydrophobic interactions between water and their hydrophobic parts [21-23]. It is believed that as the self-association proceeds, the Solvent Accessible Surface Area (SASA) of the hydrophobic solute becomes constantly limited in a linear correlation with the unfavourable contacts [21, 24-25]. Figure 2 displays the SASA profile to quantify the progress of BCNU self-association with PEG-PCL2 nanoparticles over the whole span of simulation time.

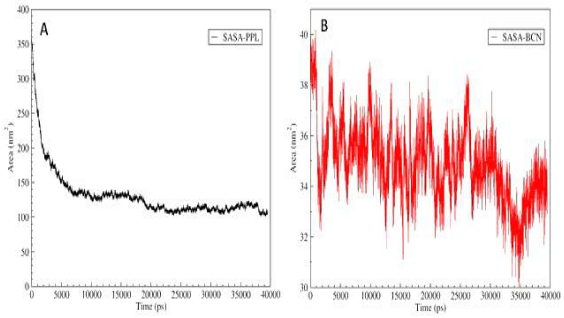

Fig. 2. Comparison of solvent accessible surface area (SASA) of (A): PEG-PCL2; (B): BCNU; PPL: PEG-PCL2; BCN; BCNU

As seen in Figure 2A, SASA is around 350 nm at the beginning. Then, it exhibits a significant decline to 200 nm during the first 3 ns of simulation, followed by a slower decrease reaching 150 nm until 10 ns. Next, it fluctuated around the same value towards the end of the simulation. Figure 2B illustrates solvent-

accessible surface area of micelles. At first, the SASA of BCNU was about 39 nm, dropped to 35 nm in the next 3 ns and then fluctuated around the average value of 35 nm until 30 ns. A final 3 nm drop was observed at the terminal stages of the simulation. To gain a further understanding of the penetration of the drug into micelles, the density of the drug inside the micelles was evaluated (Figure 3).

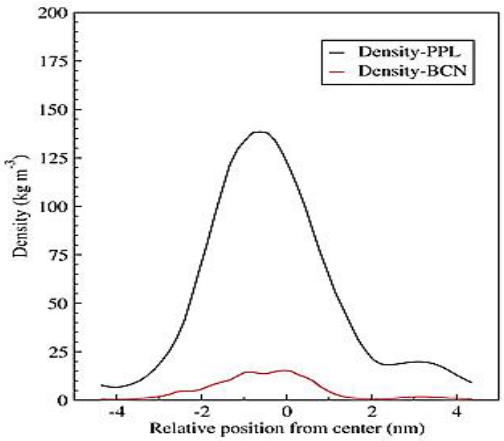

Fig. 3. Densities of the drug (red) and micelles (black) monomers.

In Figure 3, density distributions of BCNU (red) and PEG-PCL (black) are plotted as a function of distance from the micelle center of mass. The plot confirms deep penetration of BCNU into micelles.

The thermodynamic parameters of binding reaction could be used as the main evidence for confirming the interaction between micelle and drug. Electrostatic and Van der Waals interactions and hydrogen bonds (H-bond) are three major types of interactions between the micelles and the drugs. Electrostatic and Van der Waals energies and H-bond interactions were shown in Figure 4.

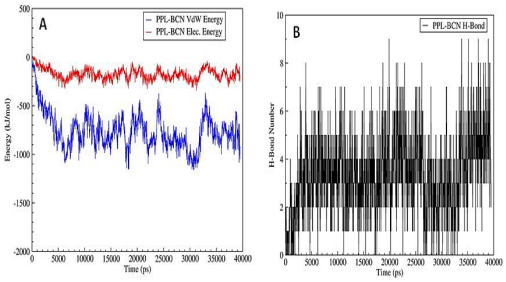

Fig. 4. (A): Van der Waals (red) and electrostatic energy (blue) between PEG-PCL2 and BCNU; (B): Amount of H-bonds formed between PEG-PCL2 and BCNU. PPL-BCN: PEG-PCL2-BCNU

AS observed in Figure 4A, red and blue curves depict electrostatic and van der Waals interactions, respectively. Their energies started at zero, after 5 ns van der Waals values decreased significantly to -1000 KJ/mol, then had minimal fluctuations until the end of simulation. In regards to electrostatic energy, it declined to -200 KJ/mol during the first 10 ns of simulation and remained unchanged. This suggests that van der Waals interactions play an effective role in the penetration of BCNU into micelle. Investigation of the hydrogen-bond formation between micelle and drug (Figure 4B) revealed that number of hydrogen bonds increased during simulation and reached 7 bonds at the end of simulation. The hydrogen bonds formed between the micelle and drug are shown in Figure 5.

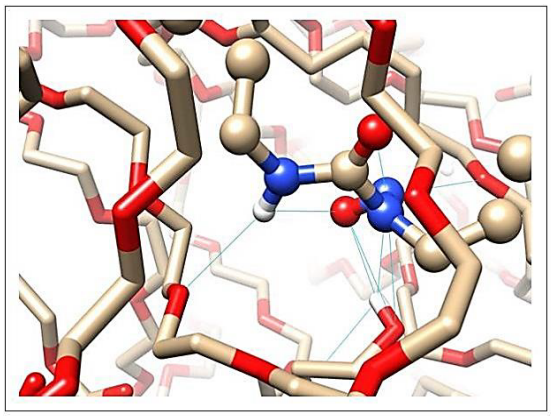

Fig. 5. Snapshot of hydrogen bond network (blue lines) between PEG-PCL2 and BCNU. BCNU participating in this hydrogen bond network is showed in the ball-and-stick models

Coarse-grained

In the coarse-grained simulation, two different systems with MePEG17-b-PCL2 (PEG-PCL2) were modelled: system I consisted of 60 monomer molecules in presence of 15 BCNU molecules in an aqueous medium. System II were formed with 300 micelle monomers and 50 drug molecules in aqueous medium.

Micelle structure

PCLs hydrophobic part formed the micelle core and PEGs (hydrophilic part) the shell. The spontaneous sphere aggregation process of BCNU into PEG-PCL2 is illustrated in Figure 6, PEG head groups and PCL tails shown in red and green, respectively, whereas BCNU is in yellow, all designed by VMD program. As represented in Figures 5B and 6A BCNUs penetrated into micelles (until the end of hydrophilic chains (PEG) consistent with our all-atom simulation results. BCNUs are drawn into the micelles; water molecules are not shown for clarity.

Our results in Figure 6 show that 60 monomer micelle generated a spherical micelle while micelle with 300 monomers formed a worm-like micelle. Lovede and associates have reported micellar shape changes from a sphere to a worm as the number of monomers rise up [26].

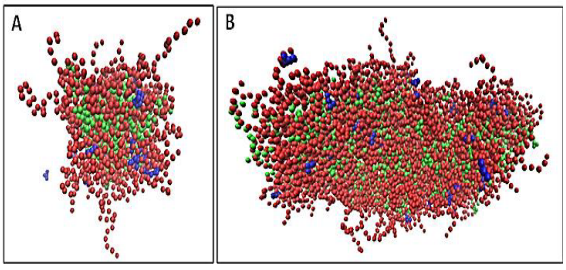

Fig. 6. Snapshot pictures during molecular dynamics (MD) simulations of PEG-PCL2 with (A): 60; (B): 300 polymers

Radial distribution function

The Radial Distribution Function (RDF), typically called g(r), in the range r0 to rmax was used to calculate the interatomic interactions in order to determine the structural properties of

the self-assembled structures. They represent the average density of varied chemical groups of the micelle as a function of their distance from the micelle's center of mass. Figure 7 displays the RDFs of PEG-PCL2 in presence of BCNUs against water.

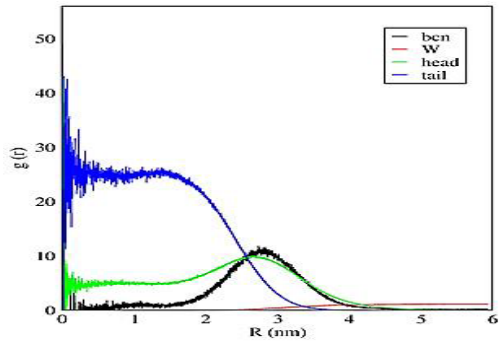

Fig. 7. Radial distribution function for the various coarse-grained particle types with water particles for the single PEG-PCL2 in water. Bcn: BCNU (black); W: water (red)

As seen, PEG (hydrophilic head) and PCL (hydrophobic tail) chain shown in green and blue, respectively. Core of the micelle had higher concentration of hydrophobic chains, whereas BCNU did not demonstrate high intensity in micelle's core. The peak of the drug was at 3 nm from the micelle's center of mass. BCNU mean density is at the PEG region close to the micelle core at the boundary between hydrophobic and hydrophilic regions of nanoparticles. This provides a precise estimation of the depth of penetration of BCNUs into the micelle. Water thickness at the interior region of the micelle is close to 0 and water density increases with the rise in micelles' radii.

CONCLUSION

All-atom and coarse-grained MD simulations have been used to investigate the penetration of BCNU molecules into PEG-PCL nanoparticles. Radius of gyration and SASA results revealed a rapid and stable self-assembly of nanoparticles and penetration of BCNUs into the micelles in aqueous solution. Based on observed results, Van der Waals and electrostatic interactions play important roles in the binding interaction between BCNUs and PEG-PCL micelle. Also numbers of hydrogen bond were evaluated; the intermolecular hydrogen bonds can be considered as the driving forces responsible for the stabilization of the studied complexes. The number of hydrogen bonds reached 7 until the end of the simulation. Coarse grain self-assembly for PEG-PCL was investigated by studying structural properties of two separate systems in water. Sphere micelle composed of 60 monomer molecules in presence of 15 BCNU and copolymer with 300 monomers and 50 BCNU constituted a worm-like micelle when placed in a cubic simulation box of water for 500 ns. These structural and dynamical findings indicate that the PEG-PCL nanoparticles have carrier potentials for BCNU molecules.

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