European Journal of Pharmaceutical Sciences 103 (2017) 128–135

Contents lists available at ScienceDirect

European Journal of Pharmaceutical Sciences

journal homepage: www.elsevier.com/locate/ejps

A computational study suggests that replacing PEG with PMOZ may increase exposure of hydrophobic targeting moiety

Aniket Magarkar[a,b], Tomasz Róg[c], Alex Bunker[a,*]

[a] Drug Research Program, Faculty of Pharmacy, University of Helsinki, Helsinki, Finland
[b] Institute of Organic Chemistry and Biochemistry, Academy of the Sciences of the Czech Republic Prague, Czech Republic
[c] Department of Physics, University of Helsinki, Helsinki, Finland

ARTICLE INFO

Article history:
Received 23 December 2016
Received in revised form 28 February 2017
Accepted 7 March 2017
Available online 9 March 2017

Keywords:
Polyoxazolines
Targeted delivery
PEGylation
Liposome
Nanomedicine
Molecular dynamics

ABSTRACT

In a previous study we showed that the cause of failure of a new, proposed, targeting ligand, the AETP moiety, when attached to a PEGylated liposome, was occlusion by the poly(ethylene glycol) (PEG) layer due to its hydrophobic nature, given that PEG is not entirely hydrophilic. At the time we proposed that possible replacement with a more hydrophilic protective polymer could alleviate this problem. In this study we have used computational molecular dynamics modelling, using a model with all atom resolution, to suggest that a specific alternative protective polymer, poly(2-methyloxazoline) (PMOZ), would perform exactly this function. Our results show that when PEG is replaced by PMOZ the relative exposure to the solvent of AETP is increased to a level even greater than that we found in previous simulations for the RGD peptide, a targeting moiety that has previously been used successfully in PEGylated liposome based therapies. While the AETP moiety itself is no longer under consideration, the results of this computational study have broader significance: the use of PMOZ as an alternative polymer coating to PEG could be efficacious in the context of more hydrophobic targeting ligands. In addition to PMOZ we studied another polyoxazoline, poly(2-ethyloxazoline) (PEOZ), that has also been mooted as a possible alternate protective polymer. It was also found that the RDG peptide occlusion was significantly greater for the case of both oxazolines as opposed to PEG and that, unlike PEG, neither oxazoline entered the membrane. As far as we are aware this is the first time that polyoxazolines have been studied using molecular dynamics simulation with all atom resolution.

1. Introduction

Nanomedicine (Debbage, 2009; Haberzettl, 2002), the development of nanoscale drug delivery vehicles, known as “nanovectors” (Riehemann et al., 2009) that encapsulate, protect and, in some cases, target a drug payload, is one of the most promising current avenues of drug research. Several different forms of nanovector have been proposed (Loomis et al., 2011; Misra et al., 2010), including solid nanoparticles (Doane and Burda, 2012), polymeric micelles (Kataoka et al., 2012; Lavasanifar et al., 2002; Sanna et al., 2014; Yokoyama, 2014) and dendrimers (Gajjar et al., 2015; Svenson and Tomalia, 2012). By far the most successful so far, however, is the liposome based delivery system (LDS) (Bunker et al., 2016); more trials of LDS based therapies are currently underway than all other forms of nanovector combined (Etheridge et al., 2013).

A liposome is a lipid membrane formed into an enclosed pocket. Through several possible modifications, it can play the role of a drug delivery vehicle: an LDS. As such it is a highly versatile carrier, capable of carrying hydrophobic drugs in the lipid membrane, or hydrophilic drugs within the internal aqueous pocket. Through formulation, e.g. inclusion of cholesterol, the lipid membrane structure can be optimized. Bloodstream lifetime can be prolonged through inclusion of a protective polymer corona “stealth sheath”, attained through inclusion of lipids with the polymers conjugated to their headgroups. The current gold standard for protective polymer is poly (ethylene-glycol) (PEG); when PEG is used as the protective polymer corona the liposome is said to be “PEGylated”. The very first approved LDS based drug therapy, Doxil® (Barenholz, 2012; Gabizon et al., 1994), is composed of distearoylphosphatidylcholine (DSPC), cholesterol (CHOL) and the lipids functionalized to PEG2000 (two kilodalton PEG; 45 monomer units long) to produce PEGylation, distearoylphosphatidylethanolamine-polyethylene glycol (DSPE-PEG). The molar ratio of DSPC:CHOL:DSPE-PEG in Doxil® is 66:33:5.

* Corresponding author.
E-mail address: alex.bunker@helsinki.fi (A. Bunker).

http://dx.doi.org/10.1016/j.ejps.2017.03.008
0928-0987/

While PEG has proved extremely effective, it is by no means perfect: 1) while PEGylation has been shown to increase bloodstream lifetime from ~ 1 h to ~1–2 days (Moghimi and Szebeni, 2003), red blood cells, platelets and some antibodies circulate for 1–2 months (Gabizon et al., 1994). and 2) PEG is immunogenic (Armstrong et al., 2007; Richter and Akerblom, 1983; Środa et al., 2005; Yang and Lai, 2015) and this has recently been shown to be a significant issue (Ishida and Kiwada, 2008; Ishida et al., 2003); the search for possible alternatives remains an active field of research (Knop et al., 2010a). Several alternative polymers exist, including hydrophilic polycarbonates (Engler et al., 2015), polyvinylpyrrolidone (PVP) (Kaneda et al., 2004; Teodorescu and Bercea, 2015) and carbohydrate derivatives, e.g. hyaluronan (Qhattal et al., 2014), pullulan (Kang et al., 1997) and gangliosides (Taira et al., 2004). A particularly promising family of polymers is the polyoxazolines (Viegas et al., 2011), in particular poly(2-methyloxazoline) (PMOZ) and poly(2-ethyloxazoline) (PEOZ), which have both been approved for internal use.

Active targeting, the attachment of moieties, that target specific receptors in the cells of target tissue, to either drugs or drug carriers, is also an important new development in drug delivery (Ganta et al., 2008; Kwon et al., 2012; Peer et al., 2007; Petros and DeSimone, 2010; Scheinberg et al., 2010). This approach holds particular promise for cancer therapy, as tumour cells are known to overexpress certain receptors (Bazak et al., 2015). For the case of an LDS, active targeting can be achieved through conjugation of targeting moieties to the ends of a subset of the protective polymers or inclusion of moieties anchored to alternate lipid molecules. A targeting moiety that has seen particular success when conjugated to a PEGylated liposome is the RGD peptide (Du et al., 2007; Jiang et al., 2010; Meng et al., 2010; Temming et al., 2005; Zhao et al., 2009).

The development of new LDS based therapies still suffers from the same phenomenon found across nanomedicine: the field is far better at generating new published papers than actual new drug therapies (Venditto and Szoka, 2013). As we argue in a previously published review paper (Bunker et al., 2016), this can, at least partly, be attributed to the largely trial and error based methodologies used; we propose a way forward that involves the development of a rational design approach that makes use of computational molecular dynamics modelling to provide a unique window (Lee et al., 2009) on the LDS being developed. In previous work, we have had particular success in modelling the orientation in the membrane (Dhawan et al., 2016; Pathak et al., 2016) and interaction with the protective polymer corona (Lehtinen et al., 2012; Magarkar et al., 2014b) of targeting ligands in the LDS and thus provide insight into the relationship between molecular design and function.

In a previously published work, (Lehtinen et al., 2012) a new proposed targeting ligand, the AETP moiety, was found not to be effective when functionalized to the exterior of a PEGylated liposome, in spite of it passing phage display screening. Since the AETP moiety is more hydrophobic than other targeting ligands that had been found to be successful, including the RGD peptide (Du et al., 2007; Jiang et al., 2010; Meng et al., 2010; Temming et al., 2005; Zhao et al., 2009), it was thought that the cause of failure could have been submersion into the membrane core. Our computational modelling, repeated also in a subsequent paper with a more accurate model of the membrane that included cholesterol (Magarkar et al., 2014b), provided evidence that this was not the case: the cause of failure was rather occlusion by the protective PEG polymer corona itself, due to the fact that PEG is not a completely hydrophilic polymer (Dinç et al., 2010). While this was the end of the road for the development of this particular targeting ligand, this result provided general insight relevant to all targeting ligands that are more hydrophobic, as the same problem of its interaction with PEG could arise once again. We ended this paper by musing that the replacement of PEG with a more hydrophilic polymer could possibly alleviate this problem, as many alternatives to PEG have been proposed (Knop et al., 2010a). A particularly promising polymer has recently been proposed that is both more hydrophilic than PEG and has been approved for internal use: poly(2-methyloxazoline) (PMOZ) (Viegas et al., 2011).

Physically, the AETP moiety is no longer with us, however, we are able to resurrect this moiety as a computational model, and perform a computer simulation of its behavior when conjugated to alternate protective polymer corona on a section of liposome membrane, thus providing insight relevant to all hydrophobic targeting moieties that may not be effective when conjugated to a PEGylated liposome. We have studied the structure and behavior in a model of blood plasma (water with an NaCl concentration of 125 mM) of lipid membranes with the Doxil® formulation, and the Doxil® formulation with the PEG replaced by the two relevant polyoxazolines, PMOZ and PEOZ (Viegas et al., 2011). We have studied the structure of the two new polymer functionalized membranes to compare its structure and behavior to that of the PEGylated Doxil® membrane. We then repeated our simulations with the AETP and RGD peptides, performed for a PEGylated Doxil® membrane on our previous work, with the two targeting moieties conjugated to the new protective polymers. As expected, the exposure to the solvent of the AETP moiety was found to be dramatically increased when PEG is replaced by PMOZ. There were, however, some other, unexpected, discoveries concerning the structure and behaviour of the PMOZ and PEOZ polymer coronas and their interaction with the two moieties.

2. Methods

2.1. Simulation protocol

All of the simulation systems were built with 1,2-distearoyl-sn-glycero-3-phosphatidylcholine (DSPC), and cholesterol, with respective polymers distearoylphosphatidylethanolamine-polyethylene glycol (DSPE-PEG2000), distearoylphosphatidylethanolamine-2-methyl-2-oxazoline (DSPE-PMOZ) and distearoylphosphatidylethanolamine-2-ethyl-2-oxazoline (DSPE-PEOZ). Both DSPE-PMOZ and DSPE-PEOZ were also simulated with activated endothelium targeting peptide (AETP) and RGD targeting moieties. In all cases simulations were carried out under physiological salt concentration. Their composition is listed in Table 1. The lipid bilayer was obtained with a total of 288 lipids; that is 144 lipids in each leaflet constructed from a square from 12 rows and 12 columns. From these 5% (14 molecules) of DSPC were substituted with DSPE-polymer (PEG/PEOZ/PMOZ). The appropriate number of water molecules was added to the system to ensure that all molecules are properly hydrated, as well as through the periodic boundary in the xy-plane, so as to insure no contact between polymers across the periodic boundary. Based on the number of added water molecules, salt molecules were added at 150 mM. Additional ion molecules were added to neutralize the total charge of the simulated system. The lengths of the oxazoline polymers were set to 45 monomers to match PEG. All systems were simulated for 500 ns under NPT semi-isotropic conditions with the OPLS-AA forcefield (Jorgensen and Tirado-Rives, 1988) with the compatible TIP3 water model (Jorgensen et al., 1983) and recently developed parameters for lipids (Kulig et al., 2015; Maciejewski et al., 2014). Physiological concentration of NaCl was added to each simulated system and extra ions were added to achieve charge neutrality. Based on area per lipid of the simulated systems, the first 200 ns were regarded as the equilibration of the systems and all the analyses were performed on latter 300 ns of the trajectory. All simulations were carried out at a constant pressure of 1 bar through a Parrinello-Rahman barostat (time constant 1 ps) (Parrinello and Rahman, 1981) and temperature was maintained at 310 K using the Nosé-Hoover thermostat (time constant 0.1 ps) (Hoover, 1985; Nosé, 1984). Periodic boundary conditions with minimum image convention were used in all three