International Journal of Pharmaceutics 459 (2014) 30–39

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

The influence of lipid composition and surface charge on biodistribution of intact liposomes releasing from hydrogel-embedded vesicles

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ARTICLE INFO

Article history:
Received 5 September 2013
Received in revised form 4 November 2013
Accepted 5 November 2013
Available online 13 November 2013

Keywords:
Radiolabeled liposomes
In situ forming liposomal hydrogel
Liposome composition
Liposome charge
IP injection
Biodistribution

ABSTRACT

Mixed drug delivery systems possess advantages over discrete systems, and can be used as a strategy to design more effective formulations. They are more valuable if the embedded particles perform well, rather than using drugs that have been affected by the surrounding vehicle. In order to address this concept, different liposomes have been incorporated into hydrogel to evaluate the potential effect on the controlled release of liposomes.

Radiolabeled liposomes, with respect to different acyl chain lengths (DMPC, DPPC, or DSPC) and charges (neutral, negative [DSPG], or positive [DOTAP]) were integrated into chitosan-glycerophosphate. The results obtained from the biodistribution showed that the DSPC liposomes had the highest area under the curve (AUC) values, both in the blood (206.5%ID/g h^{-1}) and peritoneum (622.3%ID/g h^{-1}), when compared to the DPPC and DMPC formulations, whether in liposomal hydrogel or dispersion. Interesting results were observed in that the hydrogel could reverse the peritoneal retention of negatively charged liposomes, increasing to 8 times its AUC value, to attain the highest amount among all formulations.

The interactions between the liposomes and chitosan-glycerophosphate, confirmed by the Fourier transform infrared (FTIR) spectra as shifted characteristic peaks, were observed in the combined systems. Overall, the hydrogel could control the release of intact liposomes, which could be manipulated by both the liposome type and interactions between the two vehicles.

1. Introduction

Combining biomaterials and technologies to design and develop advanced mixed drug delivery systems, and effective drug formulations, seems to be an attractive field for research. In this circumstance, numerous studies have been reported in the literature dealing with the integration of polymeric drug delivery systems and drug-loaded liposomes (Chung et al., 2006; Hara and Miyake, 2001; Ionov et al., 2011; Mulik et al., 2009; Stenekes et al., 2001). This combined system was developed to complement the advantages, while avoiding the disadvantages, of both the liposomal and polymeric systems. Moreover, the release of the encapsulated drug, and consequently, its pharmacokinetic parameters, could be modified through sustained drug action (Mulik et al., 2009).

Liposomes represent one of the safest, most unique and potentially versatile vehicles used to date, with respect to a wide range hydrophilic or hydrophobic drug encapsulations, good biocompatibility, low toxicity, lack of immune system activation, and targeted delivery of drugs to the site of action (Chen et al., 2010; Immordino et al., 2006). Liposomal research has come a long way from its initial discovery, and now different liposomes are being engineered, in terms of size, lipid composition, or surface characteristics for specific applications (Banerjee, 2001). However, the application of conventional liposomes is mainly limited by their instability, short half-lives, and rapid clearance by the mononuclear phagocyte system (MPS). To circumvent these drawbacks, two polymeric approaches have been suggested so far: first, the surface modification of liposomes with hydrophilic polymers, such as polyethylene glycol (PEG), to produce MPS-evading vesicles; and second, the incorporation of liposomes within depot polymer-based systems (Mufamadi et al., 2011).

The protection of liposomes from recognition by cells of the MPS is due to the steric hindrance effects of the polymers to opsonins (Boerman et al., 2000), while liposomal polymeric systems offer the possibility of a controlled release of intact liposomes from a

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0378-5173/$ – see front matter © 2013 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.ijpharm.2013.11.011

reservoir in a sustained way (Alinaghi et al., 2013; Stenekes et al., 2001). The benefits of the latter composite system comprise an improvement in liposome stability, increased efficacy, the ability to control drug release for a longer period of time, reduced systemic toxicity, and the protection of sensitive drugs in a polymeric-based technology (Mufamadi et al., 2011). Moreover, local drug retention and sustained drug release for longer periods of time profit from creating an ideal reservoir for drugs or liposomes (Budai et al., 2007; Hurler et al., 2012; Mulik et al., 2009; Nie et al., 2011). In this regard, the in situ formation of hydrogels, because of their biodegradability, biocompatibility and nontoxicity, have been considered for delivering drugs directly to the site of action, through subcutaneous, intraperitoneal, or intratumoral injections. This highlights the valuable potential use of liposomal hydrogels as alternative strategies for topical, ocular, and chemotherapy, to improve both the efficacy and residence time of drugs in a targeted site.

Ocular drug delivery benefits from liposomal hydrogels, since ocular therapy has long been a challenging task, mainly due the insufficient residence time of liquid formulations in the conjunctival sac (Sultana et al., 2006). The effects of different polymers for gel formation and lipid composition on the in vitro release of ciprofloxacin were studied by Budai et al. (2007). Polyvinyl alcohol and polymethacrylic acid (PMA) derivatives were applied in various concentrations in the presence of vesicles from lecithin and DPPC. Among the liposomal formulations, DPPC-PMA 0.1% presented the highest ciprofloxacin half-time, indicating that both the lipid composition with respect to the saturation of the phospholipid, and the type of polymer, were responsible for the differences found in the in vitro release of the antibacterial agent.

Topical drug delivery is another area that the application of liposomal hydrogel as an alternative strategy has explored. As an example, mupirocin-in-liposomes-in-hydrogels were proposed as an advanced delivery system for improved burn therapy by Hurler et al. (2012). This formulation resulted in the prolonged release of liposomally associated mupirocin (both in vitro and ex vivo), remarkable bioadhesiveness, and antimicrobial potential effects. In addition, the drug release was affected by vesicle size, so that the hydrogel formulations containing smaller liposomes exhibited significantly lower release rates, when compared to the micron sized multilamellar vesicles.

Obviously, liposomal hydrogels are promising carriers that may be loaded with a wide spectrum of chemotherapy drugs, to be used in parenteral formulations for treating local cancers. Nie et al. (2011) developed a thermosensitive Pluronic based hydrogel containing liposomes, for the controlled delivery of paclitaxel. Due to the increased viscosity of the liposomal gel, which has the effect of creating a drug reservoir, the longest drug-release period, compared with the liposome, gel, and the commercial formulation Taxol®, was observed.

The majority of proposed mixed drug delivery systems have focused on formulations which aim to sustain the release of drugs over prolonged time. To the best of our knowledge, the current research could be differentiated from others in monitoring the escape of intact liposomes as a second vehicle from surrounded hydrogel in injection site and its fate in the body which could be affected by liposome formulation versatility. In a previous study, we focused on the release of intact radiolabeled liposomes from a chitosan β-glycerophosphate in situ forming hydrogel, and its tissue distribution after intraperitoneal injection in mice. The results showed that this system could prolong the release of liposomes in the peritoneum, and increase the durability of liposomes in the blood, compared to the liposomes or hydrogel (Alinaghi et al., 2013). It has been known for many years that the fate of liposomes in body and tissue distribution could be manipulated by liposome properties, including size, fatty acyl chain length of the phospholipids, head group compounds and charges, and the inclusion of other lipids (like cholesterol) (Charrois and Allen, 2004). In the current study, the effects of liposome compositions and surface charges, as well as the possible effects of hydrogel on the peritoneal retention and tissue distribution of embedded liposomes, have been explored, following the intraperitoneal injection of radiolabeled formulations into mice.

2. Materials and methods

2.1. Materials

1,2-Distearoyl-*sn*-glycero-3-phosphocholine (DSPC), 1,2-dipalmitoyl-*sn*-glycero-3 phosphocholine (DPPC), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE) were purchased from Lipoid GmbH (Germany), and 1,2-dioleoyl-3-trimethylammonium-propane, chloride salt (DOTAP) and 1,2-distearoyl-*sn*-glycero-3-phosphoglycerol, sodium salt (DSPG) from Northern Lipids Inc. (Vancouver, Canada). Cholesterol (chol), glutathione (GSH), and β-glycerophosphate were provided by Sigma (Germany). ChitoClear® (chitosan) with medium viscosity (400 KD), and a 95% degree of deacetylation, was obtained from Primex (Iceland). Nuclepore® polycarbonate membranes, with pore sizes of 100 and 200 nm and a diameter of 25 mm, were purchased from Whatman Int. Ltd. (Singapore, Malaysia), and chloroform, methanol, ammonium thiocyanate, $FeCl_3 \cdot 6H_2O$, KCl, NaCl, $Na_2HPO_4 \cdot 12H_2O$, and KH_2PO_4 from Merck (Darmstadt, Germany). Sephadex G-25 fine and Sephadex G-50 medium were provided by Fluka (Switzerland). HMPAO (hexamethylpropyleneamineoxime) kits containing 0.5 mg HMPAO and 5 μg $SnCl_2 \cdot 2H_2O$ were prepared in-house. ^{99m}Tc (as sodium pertechnetate; TcO_4^- Na^+), was eluted from a $^{99}Mo/^{99m}Tc$ generator.

2.2. Preparation of liposomes

Liposomes with different compositions (Table 1) (different fatty acyl chain lengths and charges) were prepared by using the thin-film hydration method, which was described previously (Alinaghi et al., 2013). For three formulations testing fluidity, a 50:45:5 molar ratio of phospholipid (DSPC, DPPC, or DMPC):cholesterol:DOPE was chosen. In the other cases, charged lipids were added at a concentration of 15 mol%. The lipids were dissolved in a mixture of chloroform/methanol (2:1, v/v) and the solvents were removed using a rotary evaporator under reduced pressure, at a temperature of 10–15 °C higher than the transition temperature of the main phospholipid. The thin layer of lipid was then hydrated in a solution of 100 mM glutathione in phosphate buffered saline (PBS) (pH = 7.4), so that the total lipid concentration was 40 mM. Multilamellar vesicles were extruded through 0.2 μm and 0.1 μm polycarbonate membrane filters at the same temperature. The removal of the unentrapped glutathione was performed by passage over a 1.1 cm × 23 cm Sephadex G-50 column.

2.3. Characterization of liposomes

The phosphatidylcholine content of the liposomes was determined using the Stewart method (1980) and the

Table 1
Types of liposomes and symbol used in the text.

Symbol	Composition	Molar ratio
DSPC	DSPC:Chol:DOPE	50:45:5
DPPC	DPPC:Chol:DOPE	50:45:5
DMPC	DMPC:Chol:DOPE	50:45:5
DSPG	DSPC:Chol:DSPG:DOPE	35:45:15:5
DOTAP	DSPC:Chol:DSPG:DOPE	35:45:15:5