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Post-insertion of poloxamer 188 strengthened liposomal membrane and reduced drug irritancy and in vivo precipitation, superior to PEGylation

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ABSTRACT

The ultimate aim of this study was to develop asulacrine (ASL)-loaded long-circulating liposomes to prevent phlebitis during intravenous (i.v.) infusion for chemotherapy. Poly(ethylene)glycol (PEG) and poloxamer 188-modified liposomes (ASL-PEGL and ASL-P188L) were developed, and ASL was loaded using a remote loading method facilitated with a low concentration of sulfobutyl ether-β-cyclodextrin as a drug solubilizer. The liposomes were characterized in terms of morphology, size, release properties and stability. Pharmacokinetics and venous tissue tolerance of the formulations were simultaneously studied in rabbits following one-hour i.v. infusion via the ear vein. The irritancy was assessed using a rat paw-lift/lick model after subplantar injections. High drug loading 9.0% w/w was achieved with no drug leakage found from ASL-PEGL or ASL-P188L suspended in a 5% glucose solution at 30 days. However, a rapid release (leakage) from ASL-PEGL was observed when PBS was used as release medium, partially related to the use of cyclodextrin in drug loading. Post-insertion of poloxamer 188 to the liposomes appeared to be able to restore the drug retention possibly by increasing the packing density of phospholipids in the membrane. In rabbits ($n = 5$), ASL-P188L had a prolonged half-life with no drug precipitation or inflammation in the rabbit ear vein in contrast to ASL solution. Following subplantar (footpad) injections in rats ASL solution induced paw-lick/lift responses in all rats whereas ASL-P188L caused no response ($n = 8$). PEGylation showed less benefit possibly due to the drug 'leakage'. In conclusion, drug precipitation in the vein and the drug mild irritancy may both contribute to the occurrence of phlebitis caused by the ASL solution, and could both be prevented by encapsulation of the drug in liposomes. Poloxamer 188 appeared to be able to 'seal' the liposomal membrane and enhance drug retention. The study also highlighted the importance of bio-relevant in vitro release study in formulation screening.

1. Introduction

Asulacrine (ASL) is an inhibitor of topoisomerase II synthesized by The University of Auckland, New Zealand [1]. It is an analogue of the anti-leukaemia drug amsacrine with a broader anti-tumor spectrum. In clinical trials ASL showed promising data in the treatment of breast and lung cancers [2,3]. However, ASL caused pain following intravenous (i.v.) infusion. A high incidence of phlebitis (53%), inflammation of the veins, was the most significant complication [3] when administered in a 5% glucose solution (0.5–1 mg/mL as isethionate salt, pH < 4.5) which hampered the further development of ASL.

Intravenous infusion of many anticancer drugs is associated with phlebitis, including bisantrene [4], doxorubicin [5], epirubicin [6], vinorelbine, mustine and 5-fluorouracil [7,8]. The mechanism for causing phlebitis is still unclear. It has been reported that drug irritancy [8], post injection drug precipitation (PIP) [9,10] and non-physiological pH of the formulation [11] were associated with the occurrence of phlebitis of i.v. infusion. Many poorly water soluble drugs have been reported to have PIP [12] and cause irritation upon injection due to the prolonged drug–tissue contact. Our previous study [13] showed that ASL has a basic pK_a of 6.7 and its aqueous solubility at pH 7.4 is only 0.8 μg/mL. The dilution of the ASL isethionate salt solution with a phosphate buffer (pH 7.4) resulted in immediate drug precipitation, indicating that PIP may have occurred.

Formulations employed to reduce drug irritancy and PIP include emulsion [14], cyclodextrin inclusion [15] and micelles [16]. Increasing

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attention has been paid on liposomes, biocompatible drug carriers, as a solution to separate the encapsulated drugs from the surrounding tissue fluid and prevent drug PIP and/or irritation by controlled release, thus protecting the vein or tissues from damage during i.v. infusion [17–19]. Additionally, liposomes have been well documented as tumor-targeted drug delivery system by exploiting the enhanced permeability and retention (EPR) effect [20], and thus leading to an improved therapeutic effect and decreased toxicity [21,22].

From the liposome formulation perspective, maximized drug loading and optimized drug retention are crucial to ensure antitumor effect [23]. In general, remote loading is considered to be more effective than passive loading for achieving higher drug content and better drug retention due to its active influx pumping mechanism and a "locking" effect for the drug inside liposomes [21]. However, the poor aqueous solubility often represents a major challenge for liposomal drug loading as a high concentration of drug solution for drug loading is essential. To this end, cyclodextrins (CD) have been recently successfully explored as a solubilizer or stabilizer for a supersaturated drug solution [24]. However, CD may destabilize liposomal membranes by forming inclusion complexes with phospholipids or cholesterol [25,26].

Coating liposomes with hydrophilic polymers, such as polyethylene glycol (PEG) or poloxamers, have been widely used as a 'stealth' technology to reduce the clearance by reticuloendothelial system (RES), extending liposomal blood circulation. It is also an effective way to reduce drug adherence to cells [27] or tissues hence avoiding drug irritation. In addition, coating liposomes with hydrophilic polymers could increase lipophilic drug retention in the carriers [28,29] by creating a barrier for drug diffusion from liposomal aqueous core at the bilayer membrane.

This study aimed to develop ASL-liposome system as an alternative formulation to prevent phlebitis following i.v. chemotherapy. To increase drug loading, a low concentration of CD was employed as a solubilizer for ASL to create a high ASL concentration gradient for remote loading using a trans-membrane ammonium sulphate gradient. PEG and poloxamer 188 were used to modify the ASL liposomes. The effect of the use of CD on drug release from both PEGylated and poloxamer 188 coated liposomes was investigated. Pharmacokinetics was evaluated following a one-hour i.v infusion in New Zealand White rabbits with ASL free solution as a control formulation. The inflammatory reaction in a rabbit ear vein was compared between different drug formulations. The irritancy of the free drug and formulations was also assessed on a rat paw-lick/lift model.

2. Materials and methods

2.1. Materials

Asulacrine isethionate salt (99% pure) was synthesized by Auckland Cancer Society Research Centre. The phospholipids, 1,2-dipalmitoyl-sn-glycero-3-phosphocholinemonohydrate (DPPC) and N-(carbonyl-methoxy-polyethyleneglycol2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE-mPEG 2000) were purchased from Lipoid GmbH (Ludwigshafen, Germany) and 1,2-dioctadecanoyl-sn-glycero-3-phosphoethanolamine (also known as 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, DSPE) from Avanti Polar Lipids, Inc. Cholesterol was obtained from Sigma-Aldrich Co., Ltd. Sulfobutyl ether-β-cyclodextrin (SBE-β-CD, Captisol®) was a gift sample from Captisol Technology (La Jolla, USA). Poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol) (poloxamer 188), was kindly donated by BASF (Auckland). Methanol and acetonitrile were of chromatographic grade and all other reagents were of analytical grade.

2.2. Animals

All the animals used were obtained from Vernon Jansen Unit (VJU) of The University of Auckland. The experiments were approved by the Committee on Animal Experiments of The University of Auckland (Ethic No. C881). New Zealand White rabbits weighing 3.0-3.5 kg and male weanling Sprague–Dawley rats weighing 180–220 g were used and maintained according to the standards relating to the care and management of experimental animals of New Zealand.

2.3. Preparation of modified ASL liposomes

2.3.1. Plain liposomes

To prepare plain liposomes of ASL (ASL-L), DPPC, DSPE and cholesterol (mole ratio = 6:1:3, total lipids 20 mg/mL) were dissolved in chloroform: methanol (3:1, v/v) and evaporated to form a thin lipid film in around-bottom flask under vacuum condition using a rotatory evaporator (R-215, Büchi, Switzerland). The thin film was then hydrated with a 250 mM ammonium sulphate solution followed by seven cycles of freeze–thaw. Thereafter, the liposome suspension was extruded through 0.2 μm followed by 0.1 μm pore size polycarbonate membranes (Whatman, UK) with a LIPEX™ Extruder (Northern Lipids Inc, Burnaby, Canada) to obtain unilamellar liposomes. The extra-vesicular ammonium sulphate was removed through dialysis against an iso-osmotic NaCl solution for 20 h (100 mL for 1 mL liposomes each time, 4 times) at 37 °C. Then liposome suspension was incubated with same volume of drug supersaturated solution containing 5% SBE-β-CD at 37 °C for 1.5 h. Then a low speed centrifuge at 700 × g for 10 min was applied to remove the untrapped drug precipitate (if any) followed by an immediate ultracentrifuge at 188, 272 × g at 4 °C for 1 h to isolate the drug loaded liposomes from the supernatant containing SBE-β-CD and free drug. All the liposomal pellets were stored at 4 °C in the dark for later studies.

2.3.2. Poloxamer 188 coated liposomes (ASL-P188L)

Two methods were compared to obtain ASL-P188L: pre-insertion [30] and post-insertion [31]. For the pre-insertion method, a same thin-film was prepared as described above, but hydrated with 250 mM ammonium sulphate solution containing poloxamer 188 (poloxamer 188/lipids weight ratio = 1:14, molar ratio ~6%). The following procedures were the same as for ASL-L described above. For the post-insertion method, ASL-L were incubated with a poloxamer 188 solution for 30 min at 45 °C, above the phase transition temperature of lipids (42 °C). Then the liposomes were ultracentrifuged to remove the unbound poloxamer 188. Any drug precipitate formed during post-insertion should be removed using low speed centrifuge. ASL-P188L with optimal properties prepared by one of the methods was chosen for the later studies.

2.3.3. PEGylated liposomes (ASL-PEGL)

With the pre-insertion method, DSPE-mPEG 2000 was added to other lipids and the procedures and formula were same as for ASL-L described above, with only DSPE replaced with DSPE-mPEG 2000 at the same mole ratio. Post-insertion of the same mole ratio of DSPE-mPEG 2000 was conducted using the same method described for ASL-P188L. More stable ASL-PEGL prepared by one of the methods was chosen for the later studies.

2.4. Characterization of liposomes

To view the lamellarity of liposomes and the drug existing state in liposomes, liposomes with or without drug were observed using cryo-transmission electron microscopy (cryo-TEM). Briefly, a drop of each sample (theoretical lipid concentration 10 mg/mL) was placed on the copper grid containing a polymer film in the climate chamber and blotted, forming a thin aqueous layer on the membrane. The samples were shock-frozen by dipping into liquid ethane and cooled to 90 K by liquid nitrogen. The copper grid containing the sample was transferred to the Tecnai 12 electron microscope (FEI, Hillsboro, USA) operating at 120 KV where it analyzed.