International Journal of Pharmaceutics 473 (2014) 326–333

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

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

Pharmaceutical nanotechnology

The influence of phospholipid on the physicochemical properties and anti-tumor efficacy of liposomes encapsulating cisplatin in mice bearing C26 colon carcinoma

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ARTICLE INFO

Article history:
Received 8 May 2014
Received in revised form 16 July 2014
Accepted 16 July 2014
Available online 19 July 2014

PubChem classifications:
Cholesterol (PubChem CID: 5997)
Chloroform (PubChem CID: 6212)
Cisplatin (PubChem CID: 2767)
Hydrochloric acid (PubChem CID: 313)
Isopropanol (PubChem CID: 3776)
Ethylenediaminetetraacetic acid
?(PubChem CID: 6049)
Methylphenazonium methosulfate
(PubChem CID: 9285)
Nitric acid (PubChem CID: 944)
Sodium chloride (PubChem CID: 5234)
Triton X-100 (PubChem CID: 5590)

Keywords:
Cisplatin
Liposome
Phase transition temperature
Cancer treatment
C26 colon carcinoma

ABSTRACT

SPI-077, cisplatin stealth liposome, is the best illustration of poor cisplatin release from liposomes and the subsequent negligible therapeutic activity. For this reason, optimizing drug release kinetics is desirable. In this report, cisplatin was encapsulated in liposomes composed of different phosphatidylcholines with various phase transition temperatures (T_m) (HSPC, DPPC, DMPC, soy phosphatidylcholine (SPC)), cholesterol and $mPEG_{2000}$–DSPE. In vitro cytotoxicity studies indicated that lowering T_m of lipids increases cisplatin release; the highest cytotoxicity was observed in SPCs. Cisplatin plasma concentration was also sensitive to the transition temperature. The highest platinum concentration observed after treatment with HSPC and DPPC liposomes, whilst the lowest was observed with SPC. HSPC and DPPC containing liposomes showed the highest therapeutic efficacy and survival with DPPC exhibited better efficacy in mouse model of C26. It seems that DPPC with T_m (41.5 °C) nearly, or close to body temperature maintains good drug retention in blood circulation. Upon extravasation through permeable tumor microvasculature, it gradually releases its payload in the tumor area better than HSPC, with a greater T_m of 55 °C. Our data suggests, the choice of T_m for lipid mixture directed to a considerable extent the rate of cisplatin elimination from plasma and therapeutic effects.

1. Introduction

Cisplatin, a well-known anticancer agent, is being used worldwide mainly in the treatment of epithelial associated malignancies (Johnsson et al., 1996; Levin and Hryniuk, 1987; Stathopoulos, 2010). Although very effective, the dose limiting adverse effects of cisplatin including nephrotoxicity, peripheral neuropathy and ototoxicity hamper dose increment and impede its extensive clinical use (Pinzani et al., 1994; Vekris et al., 2004; Yao et al., 2007).

Over the years, numerous attempts have been made to develop cisplatin analogues with an improved therapeutic index. However, the problem of toxicity and resistance persist and neither of them proved superior to cisplatin, justifying the demand of advanced formulations of the drug (Boulikas and Vougiouka, 2004; Hamelers et al., 2006; Murray et al., 1997; Weiss and Christian, 1993).

Abbreviations: T_m, phase transition temperature; AAS, atomic absorption spectroscopy; GTA, graphite tube atomizer; CP, cisplatin; MTS, [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt.

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http://dx.doi.org/10.1016/j.ijpharm.2014.07.020
0378-5173/

Site specific drug delivery vehicles including liposomes were then recognized to increase drug reaching to the tumor area using EPR effect, sometimes called passive targeting (Maeda, 2001; Maeda et al., 2009, 2013). By taking advantage of the large gaps (180–800 nm) between adjacent endothelial cells, liposomes extravasate into the tumor interstitial spaces and due to the impaired lymphatic drainage (Jain, 1987), localization of these carriers in the tumor results in an accumulation of drugs in the tumor area, thereby reducing off-target side effects (Allen and Cullis, 2004; Lasic and Papahadjopoulos, 1995).

Following their discovery, liposomes were swiftly exploited for loading, retaining and targeting cancer drugs to the tumor areas (Barenholz, 2001; Sharma and Sharma, 1997; Torchilin, 2005). Use of liposomal cisplatin is proceeding in clinical trials since its discovery (Bandak et al., 1999; Boulikas, 2009; Harrington et al., 2001; Immordino et al., 2006; Stathopoulos et al., 2006; Zamboni et al., 2004). One example is Lipoplatin™, which is a cisplatin formulation wrapped up into liposomal vesicles of 110 nm in diameter (Boulikas, 2009; Boulikas et al., 2005; Stathopoulos et al., 2005). SPI-077, stealth cisplatin liposome, with the small diameter of approximately 110 nm, is another dosage form of cisplatin developed by Alza Corporation, Mountain View, CA, USA. Cisplatin is encapsulated in the aqueous core of this sterically stabilized liposome. The design of lipid bilayer in this formulation is based on Doxil®, doxorubicin PEGylated liposomal formulation, with the rigid bilayer composed of HSPC, cholesterol and $mPEG_{2000}$–DSPE (molecular weight 2000) (Newman et al., 1999). Phase I/II studies were run in patients with head and neck, and lung cancers and despite the promising lower toxicity profile, SPI-077 failed to demonstrate efficacy in clinical trials (Harrington et al., 2001; Meerum et al., 2002; White et al., 2006). In fact, cisplatin molecules encapsulated in the aqueous core exhibited poor dissociation from prolonged circulating liposomes which results in the subsequent negligible therapeutic activity. Thus, the combination of enhanced tumor drug delivery and optimal drug release kinetics are prerequisite for increased therapeutic efficacy (Drummond et al., 1999). To this end, investigators emphasized on optimizing the entrapped drug loss by altering the liposomal lipid composition to less penetrable vesicles to the encapsulated drug along with promoted longevity in blood (Anderson and Omri, 2004; Charrois and Allen, 2004).

Numerous studies have demonstrated that the release of the entrapped content across liposomal membrane start to be remarkable at temperatures above the gel-to-liquid crystalline phase transition temperature (T_m) (De Gier et al., 1968; Jacobson and Papahadjopoulos, 1975) which in turn, is associated with increased drug delivery at temperature above this phase transition. To explore this hypothesis, we set out to determine whether phospholipids of differing acyl chain lengths and ratios of unsaturation could alter the permeability of the drug across liposomal membrane. So, we prepared PEGylated liposomal formulations of cisplatin with phospholipids having various transition temperatures, thus, different drug release profile at body temperature, including HSPC, DPPC, and DMPC with T_m of 55, 41.5 and 25 °C, respectively, and soy phosphatidylcholine (SPC) with transition temperature below 0 °C. Besides the length of fatty acyl chain and its saturation, the proportion of cholesterol is another essential factor for maintaining liposomal drug retention (Kirby et al., 1980). Hence, another SPC containing lipid bilayer was formulated in which the cholesterol content was increased, presumably to slow release rates of cisplatin and to increase the stability of bilayer. Here we reported liposome size and zeta potential, cisplatin encapsulation efficacy and in vitro cytotoxicity of different formulations against C26 colon carcinoma. The platinum plasma concentrations of various formulations were also determined at different time points in mice. In addition, therapeutic activity and survival probability were examined in the C26 murine colon carcinoma model.

2. Methods

2.1. Materials

Hydrogenated soy phosphatidylcholine (HSPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), soy phosphatidylcholine (SPC), cholesterol (Chol) and methoxy-polyethylene glycol (MW 2000)–distearoylphosphatidylcholine ($MPEG_{2000}$–DSPE) were purchased from Avanti Polar Lipids (Alabaster, AL). Cisplatin powder was obtained from Tocris Bioscience (USA), tryptan blue, isopropanol and chloroform were purchased from Merck (Darmstadt, Germany). MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium was purchased from Promega (Madison, WI). C26 cells were purchased from Cell Lines Service (Eppelheim, Germany). Acidified isopropyl alcohol (90% isopropanol/0.075 M HCl) was prepared by addition of 7.5 ml HCl 1 M and 2.5 ml water to 90 ml isopropanol (Merck, Darmstadt, Germany). All other solvents and reagents were used as chemical grade.

2.2. Preparation of liposomes

Cisplatin loaded liposomes were prepared with various phosphatidylcholines, cholesterol and DSPE–PEG_{2000}, at 56.5:38.5:5 molar ratios. Four types of formulations were prepared with phospholipids, including HSPC, DPPC and DMPC which were all saturated neutral lipids with various lengths of acyl chains and T_m along with SPC, containing a high level of phosphatidylcholines originated from soybeans. Another SPC containing liposome with higher cholesterol content was also formulated with the following molar ratio (50:45:5). Liposomes were prepared using ethanol injection method followed by extrusion (Newman et al., 1999). Briefly, preparation of liposomes begins by dissolving lipid components (150 mM) in an absolute ethanol at 10% (v/v). Aqueous phase was made by dissolving cisplatin solution in 150 mM NaCl adjusted to pH 7.4 at 70 °C, according to its solubility. To prepare cisplatin loaded liposomes, lipid-containing ethanol phase was immediately injected into 8 mg/ml cisplatin solution through a fine needle at 70 °C. The aqueous phase rapidly became milky as a result of liposome formation. Colloidal dispersions remained at the incubation temperature for 1 h with gentle mixing every 10 min. The resulting multilamellar vesicles were sonicated for 5 min and then downsized by extrusion through stacked 1000, 400, 200 and 100 nm polycarbonate filters with a thermobarrel extruder apparatus (Northern Lipids Inc., Canada). The temperature of the samples was maintained at 70 °C to obtain the maximum solubility of cisplatin and also to make sure that the lipid compositions were well mixed in the bilayer (Zisman et al., 2011). Liposomes subsequently were dialyzed four times in 24 h against 150 mM NaCl (pH 7.4) at room temperature to remove the unencapsulated cisplatin. Sterilization of the final products was obtained by filtration using a sterile 0.45 μm filter.

2.3. Liposome characterization

The mean diameter of liposomes and polydispersity index (PDI) were determined using a Dynamic Light Scattering Instrument (Nano-ZS; Malvern, UK) in triplicate. The zeta potential of liposomes was determined on the same equipment using the zeta potential mode averaging 20 measurements. Particle sizes were reported as the means ± standard deviation and polydispersity index ($n = 6$). Zeta potentials were reported as the means ± zeta deviation ($n = 6$).