Journal of Pharmaceutical Sciences xxx (2015) 1–10

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Research Article

Preparation, Characterization, and Antitumor Activities of Miriplatin-Loaded Liposomes

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ARTICLE INFO

Article history:
Available online xxx

Keywords:
miriplatin
liposome
DMPG
HPLC
anti-tumor activity
preparation

ABSTRACT

Because of the insolubility of miriplatin in water, miriplatin and lipiodol suspension is the sole formulation of miriplatin approved in Japan to treat hepatocellular carcinoma by transcatheter arterial chemoembolization. Until now, there have been no reports of other pharmaceutical formulations of miriplatin except miriplatin/lipiodol suspension. In this study, we aimed not only to develop miriplatin-loaded liposomes (lipomiriplatins) which could be administrated systematically for tumors besides hepatocellular carcinoma but also to ascertain whether miriplatin, like its analog of NDDP, was a liposome-dependent antitumor agent. We found that miriplatin could be successfully incorporated into liposomes, and both the stability and antitumor activity of lipomiriplatins were independent of the liposomal compositions. Especially, HPLC was successfully established as the quantitative method for lipomiriplatins, which completely eliminated the interference of cholesterol. Lipomiriplatins possessed favorable colloidal properties (99.71 ± 0.56 nm, −50 mV), high drug-loading capacity (about 2.2 mg/mL), excellent entrapment efficiency (>95%), and robust stability. The remarkable antitumor activities of lipomiriplatin were proved to be mediated by inducing cell apoptosis and were comparable to that of the commercial cisplatin and oxaliplatin injections, indicating that lipomiriplatins showed great promise for future potential clinical application via systematic administration.

Introduction

Platinum-based chemotherapy plays a prominent role in the treatment of various solid tumors.[1,2] Despite being one of the most effective classes of chemotherapeutics, platinum drugs (cisplatin, carboplatin, and oxaliplatin) do have several significant drawbacks: severe side effects[3] (such as neurotoxic, myelotoxicity), short blood circulation times,[4] and the universal drug resistance.[5] Because of these limitations, there has been strong interest in the development of novel platinum-based therapeutics to not only lower toxicity but also improve therapeutic efficacy. Two main strategies are used. One is to develop new platinum analog drugs and the other is to use drug delivery technologies to engineer novel platinum drug formulations.[1,6] Liposome is a promising strategy for clinical application of platinum drugs.[7] Results from preclinical and clinical studies have shown that liposomal formulations of platinum drugs not only maintained favorable efficacy but also greatly reduced side effects, such as SPI-077 (cisplatin),[8] Lipoplatin (cisplatin),[9] Lipoxal (oxaliplatin),[10] and Aroplatin (NDDP).[11-13] Lately, Lipoplatin has finished successfully phase III clinical trials as a first-line treatment against non-small cell lung cancer in 2011[14] and has been granted phase II/III studies on pancreatic cancer as an orphan drug by the European Medicines Agency.[15] The success of Aroplatin offered us invaluable experience and great confidence of using liposomes as platinum drug formulations.

Miriplatin[16] (Fig. 1), which contains myristates as leaving groups and diaminocyclohexane (DACH) as a carrier ligand, is an analog of oxaliplatin. Lipophilic miriplatin was synthesized specifically for suspension in iodinated poppy seed oil with excellent compatibility, and the resulting miriplatin/lipiodol suspension (Miripla; Dainippon Sumitomo Pharma, Osaka, Japan) was approved in Japan to treat hepatocellular carcinoma (HCC) by transcatheter arterial chemoembolization. Until now, miriplatin/lipiodol suspension was exclusively used in transcatheter arterial chemoembolization for HCC patients approved only in Japan and was the sole formulation of miriplatin used in clinics,[17,18] which greatly hindered the clinical application of miriplatin. Moreover, the insolubility of miriplatin in water (<0.00260 mg/mL) somewhat hampers the pharmaceutical

This article contains supplementary material available from the authors by request or via the Internet at http://dx.doi.org/10.1016/j.xphs.2015.10.026.

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http://dx.doi.org/10.1016/j.xphs.2015.10.026
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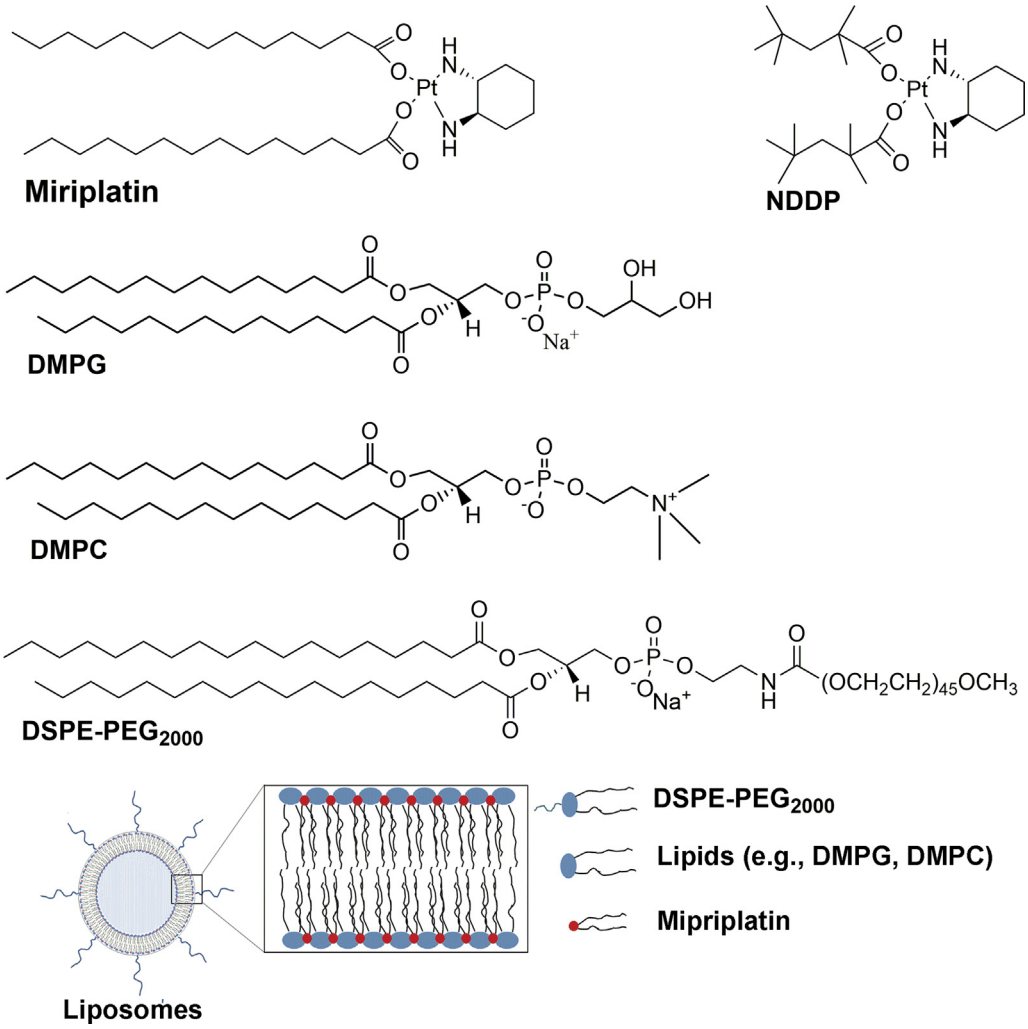

Figure 1. Chemical structures of miriplatin, DMPC, DMPG, DSPE-PEG$_{2000}$, and NDDP, and schematic diagram of miriplatin-loaded liposomes.

development of miriplatin. Given the structural similarity of miriplatin and phospholipids (Fig. 1), we hypothesized that incorporating miriplatin into liposomes might be feasible and would offer a novel formulation for miriplatin to enlarge its clinical application (e.g., treating tumors apart from HCC via systematic administration).

Aroplatin (Antigenics, Inc., Lexington, MA) is a liposomal formulation of NDDP,[11] which is structurally similar to miriplatin (Fig. 1). Although Aroplatin has reached phase II trials in colorectal neoplasms (NCT00043199), pancreatic neoplasms (NCT00081549), and malignant mesothelioma (NCT00004033, completed), its final approval could not be easily obtained. Because it was found that NDDP was a liposome-dependent antitumor agent,[19] the presence of dimyristoyl phosphatidylglycerol (DMPG) in liposome was quite essential for the activation of the prodrug NDDP to exert antitumor activities. Moreover, the degradation/activation of NDDP by DMPG took place within the liposomes and was influenced by complex factors (such as acidic pH, a high temperature, the presence and amount of acidic phospholipids, and the presence of residual chloroform),[20] which makes it quite difficult to control the conversion and keep the uniformity of the active intermediates (mainly DACH-Pt-Cl$_2$) in Aroplatin, hence casting a shadow on the further pharmaceutical development of NDDP. To develop a successful miriplatin-loaded liposome (lipomiriplatin), considering that the structures of miriplatin and NDDP are similar, it is essential for us to ascertain whether miriplatin is a liposome composition–dependent antitumor agent, the same as NDDP.

Therefore, in this study, there were 3 main objectives: (1) to develop an optimal liposomal miriplatin; (2) to verify whether miriplatin was a liposome-dependent antitumor agent; and (3) to investigate the preliminary antitumor activities of lipomiriplatin.

Materials and Methods

Materials

DMPG, DPPG [1,2-dipalmitoyl-sn-glycero-3-phospho-(1'-rac-glycerol)], DSPG [1,2-dioctadecanoyl-sn-glycero-3-phospho-(1'-rac-glycerol)], DMPC (1, 2-dimyristoyl-sn-glycero-3-phosphocholine), DPPC (1, 2-dipalmitoyl-sn-glycero-3-phosphocholine), HSPC (L-α-phosphatidylcholine), and DSPE (distearoyl phosphoethanolamine) were purchased from Shanghai Advanced Vehicle Technology Pharmaceutical, Ltd. Co [AVT]). Cholesterol (for injection) was obtained from Nippon Fine Chemical. DSPE-PEG$_{2000}$ (1, 2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino (polyethylene glycol)-2000]) was obtained from Avanti Polar Lipids, Inc. Miriplatin was bought from Kunming Guiyan Pharmaceutical Company, Ltd. Solvents and reagents of liquid chromatographic grade were obtained from E. Merck (Darmstadt, Germany). Triple deionized water