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Development of pegylated liposomal vinorelbine formulation using "post-insertion" technology

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ABSTRACT

Prolonged vinorelbine exposure is correlated with improved antineoplastic effects, as evidenced by increased response rate in patients receiving continuous infusion. The administration of slow release pegylated liposomal vinorelbine formulation might mimic the pharmacokinetics of a continuous infusion, thus improving antitumor efficacy. But it is hard to prepare pegylated liposome vinorelbine using DSPE-PEG (an extensively used peglipid) because it could induce accelerated drug release. To resolve this problem, "post-insertion" technology was employed to prepare pegylated liposome vinorelbine formulations, which involved the incubation of vinorelbine-containing vesicles with DSPE-PEG micellar solutions. HPLC analysis revealed that after incubation at 60 °C for 60 min, ~100% DSPE-PEG could be inserted into the outer monolayer of the vesicles. Moreover, the grafting of peglipid did not induce the release of entrapped vinorelbine irrespective of intraliposomal anions. Drug release experiments indicated that "post-insertion" formulations were more able to retain entrapped drugs than "co-dissolving" formulations. The same phenomenon was observed when both series of formulations were injected in normal mice to compare pharmacokinetic profiles. In L1210 ascitic model, a "post-insertion" formulation with a PEG grafting density of ~0.5% exhibited the strongest antineoplastic effects, thus it was chosen to be further evaluated in S-180 and RM-1 models, in which the formulation was still more therapeutically active than conventional formulations. In conclusion, using "post-insertion" technology, the potential interaction between DSPE-PEG and vinorelbine could be prevented, thus making it possible to develop pegylated vinorelbine formulations.

1. Introduction

Vinorelbine is a semisynthetic vinca alkaloid that is indicated as a single agent or in combination with cisplatin for the first line treatment of patients with advanced non-small cell lung cancer (NSCLC) (Jones and Burris, 1996). The vinca alkaloids are structurally similar compounds comprised of 2 multiringed units, vindoline and catharanthine. Unlike other vinca alkaloids, the catharanthine unit is the site of structural modification for vinorelbine. It has been revealed that the chemical modification resulted in altered chemophysical properties (e.g., increased lipophilicity and membrane permeability), changed toxicity and efficacy profiles relative to other vinca alkaloids (Crawford, 1996; Johnson, 1996; Johnson et al., 1996; Krikorian and Breillout, 1991; Sorensen, 1992).

All the vinca alkaloids are spindle poisons, which interfere with the polymerization of tubulin, the protein responsible for building the microtubule system that is essential to nerve conduction and also the mitotic spindle, which appears during cell division. In terms of cytotoxic action, this results in accumulation of cells at G2/M in the cell cycle (Crawford, 1996; Johnson, 1996; Johnson et al., 1996; Krikorian and Breillout, 1991; Sorensen, 1992). Due to its mechanism of action and cell cycle specificity, prolonged vinorelbine exposure is critical to achieve the optimum therapeutic effects (Aapro et al., 2001).

To realize this purpose, the employment of liposomal drug delivery system might be an option. The slow release of drugs from vesicles could guarantee the prolonged exposure of tumor cells to the released drugs. Moreover, the selective biodistribution of vinorelbine after liposome encapsulation might reduce the toxicity of vinorelbine (Chow et al., 2008, 2009; Drummond et al., 2009; Nabiev et al., 1998; Semple et al., 2005; Webb et al., 2007;

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Zhigaltsev et al., 2005, 2006). Although vinorelbine possesses relative selectivity for mitotic microtubules in comparison with other vinca alkaloids, it still exhibits other side effects in clinical practice (e.g., severe granulocytopenia) (Curran and Plosker, 2002; Gregory and Smith, 2000; Karminsky et al., 1999).

However, it is not easy to achieve liposomal vinorelbine formulation with improved therapeutic index without the development of novel strategies. Due to chemical modification, vinorelbine becomes more lipophilic and membrane permeable (Zhigaltsev et al., 2005, 2006), thus posing a challenge for effective retention of vinorelbine inside vesicles. In addition, to improve drug targeting and long-term stability, the modification of vesicles with peglipids might be indispensable, but it is reported that DSPE-PEG (an extensively used peglipid) might induce the accelerated release of vincristine from liposomes (Webb et al., 1998). Since vinorelbine is structurally similar to vincristine, the same phenomenon might occur. Therefore, to develop pegylated liposome formulation, these two obstacles must be resolved first.

In previous study, we have found that certain kinds of anions (such as 5-sulfosalicylate) could form stable aggregates with vinorelbine, thus improving vinorelbine retention. Despite that the result was encouraging, novel strategy that permits modification of vesicles with DSPE-PEG is still desirable. In this study, we mainly investigated the influences of anions, PEG grafting density and pegylation method on vinorelbine loading, release and retention. It is found that the employment of 5-ssa as trapping agents, and modification of vesicles via "post-insertion" technology could markedly improve the retention of vinorelbine. Accordingly, a pegylated liposomal vinorelbine formulation was developed, which exhibited significantly improved antitumor efficacy in L1210, S-180 and RM-1 tumor models.

2. Materials and methods

2.1. Materials

Vinorelbine bitartrate was provided by Hainan Jiamao Plant Development Co., Ltd. (Hainan, China). Hydrogenated soybean phosphatidylcholine (HSPC) was a kind gift from Degussa (Freising, Germany). N-(Carbonyl-methoxypolyethyleneglycol$_{2000}$)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine, sodium salt (DSPE-PEG) was obtained from Genzyme Pharmaceuticals (Liestal, Switzerland). Cholesterol (Chol), Sepharose 4B and Sephadex G-75 (medium) were obtained from the Sigma Chemical Company (St. Louis, MO). Nucleopore polycarbonate filters (47 mm, 0.1 μm pore sizes) were obtained from Northernlipids, Inc. (Canada). All other chemicals used in this study were analytical or high-performance liquid chromatography (HPLC) grade.

The S-180, L1210 and RM-1 tumor cell lines were originally purchased from Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences (Shanghai, China). KM mice (8–10 weeks old) were obtained from Hebei Medical University. BDF1 and c57 mice were purchased from Vitalriver (Beijing, China).

2.2. Preparation of liposomes

Liposomes were prepared according to the following procedure. Briefly, the mixtures of HSPC, Chol and DSPE-PEG were solubilized in chloroform and dried to a thin lipid film under a stream of N_2 gas, followed by incubation overnight under vacuum to remove residual solvent. In all cases, the molar ratio of HSPC to Chol was set as 3:2, but the DSPE-PEG/HSPC molar ratio might be 0, 0.5, 2.9 and 8.3%. The dried lipid films were subsequently hydrated with 300 mM desired ammonium salt solutions (e.g., sulfate, *p*-phenolsulfonate, 5-sulfosalicylate, phosphate, phytate). The hydration process was performed at 60 °C for 1 h. The dispersion was extruded eight times through polycarbonate filters of 0.10 μm employing a LiposoFast-100 jacketed extruder obtained from Avestin (Ottawa, Canada) at 60 °C. This procedure formed unilamellar vesicles of ~100 nm.

The zeta average size of vesicles was analyzed using quasi-elastic light scattering (Zetasizer Nano-ZS; Malvern Instruments, UK). Before analysis, the samples were diluted in 0.9% NaCl with a volume ratio of 1/200. The zeta potential of vesicles was also determined using Nano-ZS, but the measurement was carried out in water after 30-fold dilution. In both cases, DTS4.0 software was used to collect the data that were analyzed using "multinarrow modes".

2.3. Remote loading of liposomes

A transmembrane ammonium salt gradient was generated across the vesicles by exchanging the extraliposomal buffer using Sephadex G-75 columns. The buffer employed in the experiments was sucrose (300 mM)–histidine (20 mM) buffer (pH 7.5). Upon buffer exchange, empty liposomes with transmembrane ammonium salt gradient were mixed with concentrated vinorelbine solutions (10:1, v/v), resulting in a desired mass ratio. The resulting mixture was incubated at 60 °C for 40 min to realize drug loading. After loading, the liposomal preparations were concentrated to a vinorelbine concentration of 2 mg/mL using a Millipore Labscale TFF System (with 50,000 nominal molecular weight limit polysulfone filters).

For determining the loading efficiency, samples of the mixtures were taken and unentrapped vinorelbine was removed by size exclusion chromatography. Briefly, 100 μL samples were loaded onto Sephadex G-75 mini-column (56 mm × 8 mm i.d.), and then eluted using 0.9% NaCl solution.

2.4. Modification of vesicles with peglipids via "post-insertion" (Allen et al., 1991; Iden and Allen, 2001; Moreira et al., 2002)

Prior to experiments, liposomes and a concentrated micellar DSPE-PEG were first equilibrating to 60 °C. The insertion of peglipids into vesicles was started by mixing aliquots to give a final theoretical 0.5, 2.9 and 8.3 mol% of DSPE-PEG to HSPC in the outer monolayer. After incubation of the mixtures at 60 °C for 60 min, the micelles were separated from the liposomes by Sepharose 4B column chromatography. Liposome fraction in the void volume was collected for lipid analysis.

2.5. Determination of lipid compositions

A Waters HPLC system was used to determine lipid contents (HSPC, peglipid and cholesterol). The system was composed of 2690 liquid chromatograph and 410 RI detector, and controlled by Millennium 32 software. For the measurement of lipid content, a zobax c18 (25 cm × 4.6 mm i.d., 5 μm particle size) was employed, which was maintained at 35 °C during the analysis procedure. The mobile phase was a mixture of methanol, tetrahydrofuran (THF) and 0.17 mol/L ammonium acetate (94:5:1), running at a flow rate of 1 mL/min. The retention times for different components were 4.8 min (DSPE-PEG), 8.6 min (cholesterol), 10.1 min for the first peak of HSPC (PSPC) and 15.3 min for the second peak of HSPC (DSPC).

Two milliliters of samples were diluted to 10 mL using chloroform–methanol (1:2) mixture, and then the resulting solutions were injected into HPLC with injection volume of 10 μL for the analysis of lipid compositions.