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Preparation and evaluation of PEGylated phospholipid membrane coated layered double hydroxide nanoparticles

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ABSTRACT

The aim of the present study was to develop layered double hydroxide (LDH) nanoparticles coated with PEGylated phospholipid membrane. By comparing the size distribution and zeta potential, the weight ratio of LDH to lipid materials which constitute the outside membrane was identified as 2:1. Transmission electron microscopy photographs confirmed the core-shell structure of PEGylated phospholipid membrane coated LDH (PEG-PLDH) nanoparticles, and cell cytotoxicity assay showed their good cell viability on Hela and BALB/C-3T3 cells over the concentration range from 0.5 to 50 μg/mL.

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1. Introduction

Layered double hydroxides (LDHs) constitute a broad family of lamellar solids which in the last decades have deserved an increasing interest because of their applications in different fields, such as catalysts [1], traps for anionic pollutants [2], and flame retardants [3]. In recent years, LDHs are gaining increasing attention as carriers for drug/gene delivery [4]. They are sometimes named as anionic clays due to the similarities shared with cationic clays, or hydrotalcite-like materials, as derived from the natural hydroxycarbonate of Mg and Al [5].

LDHs are a class of anionic lamellar compounds made up of positively charged brucite-like layers with an interlayer gallery containing charge compensating anions and water molecules [6]. The LDH composition can be expressed in a general formula $[M^{2+}_{1-x}M^{3+}_{x}(OH)_2](A^{-}_{x}\cdot nH_2O)$, where M^{2+} and M^{3+} can be most divalent and trivalent metal ions and A^- any type of anions. Many studies demonstrated that anticancer drugs [7–9] and genes [9–11] could be successfully intercalated into the LDH layers.

As a nanocarrier, LDH shows many advantages over other delivery systems [4]. First, they tend to exhibit low cytotoxicity, even at a high dose. In addition, LDH is easily degraded in the acidic environment, thus endowing it an advantage of biodegradability, which is also responsible for their endolysosome escaping behavior. However, positively charged LDH nanoparticles allow avid association with the negatively charged plasma proteins, which adversely influences their pharmacokinetic behavior and reduces their blood residence time. Besides, we observed that they can easily cause death during intravenous injection.

Phospholipids have long been perceived as safe materials to compose drug delivery vehicles because of their superior biocompatibility. They could cover on the surface of other solid nanoparticles [12], and several lipid-coated hybrid carriers have been developed [13]. Very recently, we reported a PEGylated phospholipid membrane coated LDH (PEG-PLDH) delivery system with a core-shell structure [14]. This new composite system showed enhanced therapeutic efficacy and survival rate when compared to naked LDH nanoparticles since the positive charges were shielded by phospholipid membrane. DOPA was chosen as a membrane material because it readily forms liposomes [15], and has a negatively charged phosphatidic acid headgroup which aids in the shielding of positively charged LDH. A PEG–lipid conjugate was also included in the leaflet lipids to prolong the circulation time [12]. The pharmacokinetic study and *in vivo* antitumor activity of PEG-PLDH nanoparticles have been investigated; here we report the preparation, properties, as well as *in vitro* cell cytotoxicity of this novel delivery system.

2. Materials and methods

2.1. Materials

Dioleoylphosphate (sodium salt) (DOPA), distearoylphosphatidylethanolamine- [methoxy(polyethylene glycol)-2000] (DSPE-PEG_{2000}) and cholesterol (Chol) were purchased from Avanti Polar Lipids (Alabaster, AL, USA). Dilauroylphosphatidylcholine (DLPC), dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphocholine (DPPC) and distearoylphosphocholine (DSPC) were purchased from Genzyme (Cambridge, MA, USA). Methotrexate (MTX) was provided by Amresco (USA). HeLa and BALB/C-3T3 cells purchased from the Laboratory Animal Center of Sun Yat-sen University (Guangzhou, China) were cultured with DMEM medium (Sigma-Aldrich, USA) supplemented with 10% calf serum (Life Technologies, USA).

2.2. Preparation of LDH and LDH-MTX nanoparticles

LDH nanoparticle suspension was prepared with a quick precipitation and subsequent hydrothermal treatment [16,17]. In brief, 3.0 mmol of $MgCl_2$ and 1.0 mmol of $AlCl_3$ were dissolved in 10 ml deionized water. This salt solution was then rapidly added to a basic solution (30 ml) containing 6.0 mmol of NaOH within 5 s to generate the precipitate of LDH. After being stirred for 10 min in N_2 stream at room temperature, the precipitate was collected via centrifugation and further washed twice. Henceforth, the washed precipitate was manually dispersed in 20 ml of deionized water and placed in a 25 ml autoclave with Teflon linen, followed by hydrothermal treatment at 100 °C in an oven to get the suspension of LDH nanoparticles. To achieve methotrexate loaded LDH (LDH-MTX) nanoparticles, 0.1 mmol of MTX was dissolved in NaOH before the quick precipitation step.

2.3. Preparation of PEG-PLDH and PEG-PLDH-MTX nanoparticles

PEG-PLDH or PEG-PLDH-MTX nanoparticle suspension was prepared by self-assembly. Lipid materials were dissolved in $CHCl_3$ and dried under a N_2 stream. A trace amount of chloroform was removed by keeping the lipid film under a vacuum. The lipid film was hydrated with PBS (pH 7.4) to obtain an empty liposome suspension. LDH or LDH-MTX nanoparticle suspension was added to the liposomes. The mixture was sonicated (in a water bath) using a laboratory ultrasonic cleaning machine (SB-5200DTN, Ningbo Scientz Biotechnology Co., Ltd. Zhejiang, China) at 250 W.

2.4. Size and zeta potential

The average hydrodynamic diameter, polydispersity index (PdI) and zeta potential of the nanoparticles were determined by dynamic light scattering using a Malvern Zetasizer Nano ZS90 (Malvern Instruments, Worcestershire, UK). The temperature of the cell was kept constant at 25 °C. The zeta potential was calculated from the electrophoretic mobility using the Smoluchowski equation. Samples of the prepared complexes were diluted in distilled water and were measured at least three times. Size results are given as intensity distribution by the mean diameter with its standard deviation.

2.5. Transmission electron microscopy (TEM)

The particle morphology of LDH and PEG-PLDH was confirmed by using TEM. The samples were put on carbon formvar coated grids, negatively stained with uranyl acetate 1.5%, and observed using a JEOL JEM-1400 instrument (JEOL, Japan) (120 kV).