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Erythrocyte membrane nanoparticles improve the intestinal absorption of paclitaxel

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ABSTRACT

Paclitaxel (PTX) is a cytotoxic chemotherapy drug with encouraging activity in human malignancies. However, free PTX has a very low oral bioavailability due to its low aqueous solubility and the gastrointestinal drug barrier. In order to overcome this obstacle, we have designed erythrocyte membrane nanoparticles (EMNP) using sonication method. The permeability of PTX by EMNP was 3.5−fold (P_{app} = 0.425 nm/s) and 16.2−fold (P_{app} = 394.1 nm/s) higher than free PTX in MDCK-MDR1 cell monolayers and intestinal mucosal tissue, respectively. The *in vivo* pharmacokinetics indicated that the AUC_{0-t} (μg/mL·h) and C_{max} (μg/mL) of EMNP were 14.2-fold and 6.0-fold higher than that of free PTX, respectively. In summary, the EMNP appears to be a promising nanoformulation to enhance the oral bioavailability of insoluble and poorly permeable drugs.

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1. Introduction

Paclitaxel (PTX) is a major active extract ingredient in the bark of the Pacific Yew Tree (*Taxus brevifolia*), which was first obtained in a pure form and identified by Drs. Wall and Wani [1]. As a member of the taxane class, PTX is well-known for its favorable chemotherapeutic activity against various cancers, such as non-small-cell lung cancer, ovarian cancer, breast cancer, and so on [2]. PTX is a microtubule-stabilizer which combines with tubulin and then inhibits the disassembly of microtubules [3]. When the microtubule dynamics are disrupted, PTX blocks cells in the G2/M phase of the cell cycle, and then ultimately result in apoptosis.

For clinical use of chemotherapy, PTX is commonly formulated and intravenously administrated to patients. The three classic formulations under the trade name Taxol®, Abraxane® and Lipusu® have been used in clinical for many years. This kind of administration brings about higher peak plasma concentration than the maximum tolerable concentration and then rapid excretion of the drug from the circulation system. In addition, the drug excipients injected into the blood vessels directly are also reported as causing frequent side effects including hypersensitivity, neurotoxicity, and so on [4]. Therefore, lots of efforts have been focused on exploring novel PTX formulations by oral route. Oral chemotherapy could sustain a moderate drug concentration in the circulation to avoid high peak above the maximum tolerable concentration and obtain an extended exposure of cancer cells to drugs. The patients themselves can take the drug anywhere through this easy way. The oral administration extremely decreases their medical expenses and improves compliance as well as life quality of the patients [5]. Oral chemotherapy can provide a palliative treatment option for cancer patients through reducing the pain of medical intervention and increasing the hope of prolonged life. However, the oral application of PTX is mainly limited by its low aqueous solubility (less than 0.4 μg/mL), poor permeability and low absorption [6]. The

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multidrug efflux proteins, i.e. P-type glycoproteins (P-gp), which are rich in the epithelial cell membranes in the gastrointestinal tract greatly reduce the absorption of PTX.

Fortunately, nanomedicine may provide an ideal solution for these limitations. Several PTX nano-formulations have shown tremendous potential to improve oral bioavailability [7–9]. Nevertheless, the preparations of many nano-formulations were complex, and some of them were mixed with several materials which may affect humans' health. In this case, we turned our attention to the erythrocyte membrane drug delivery system.

Erythrocyte membrane have been explored as drug carriers for more than three decades [10,11]. With the development of biology and nanomedical science, they have received growing interest as a promising drug delivery system recently [12–14]. Compared with other artificial nanocarriers, erythrocyte membrane drug delivery system was thought to have more desirable advantages, including intrinsically biocompatibility, non–immunogenicity, simple manufacture and extensive nature sources. What's more, this kind of carriers has a well-known amphiphilic double phospholipid membrane structure, which can carry hydrophilic or hydrophobic drugs. The homologous phospholipid membrane structure has strong similarities to intestinal membrane with regard to composition, which could be served as a clue to promote the oral delivery of the hydrophobic PTX [14].

In this research, we designed an erythrocyte membrane nanoparticles (EMNP) using a sonication method. The physicochemical properties of EMNP were characterized. Compared with the free PTX, EMNP showed 3.5–fold (P_{app} = 0.425 nm/s) improved permeability of PTX in MDCK-MDR1 cell monolayers and 16.2–fold (P_{app} = 394.1 nm/s) improved permeability in intestinal mucosal tissue *in vitro*. The cell uptake experiment also showed efficient cellular uptake of the hydrophobic drug in MDCK-MDR1 cell monolayers, suggesting the increased facilitated uptake through endocytic pathways. *In vivo*, the pharmacokinetics indicated that the AUC_{0-t} (μg/mL·h) and C_{max} (μg/mL) of EMNP was 14.2-fold and 6.0-fold higher than that of free PTX, respectively. In summary, the EMNP appears to be a promising nanoformulation for insoluble and poorly permeable drugs to enhance the oral bioavailability.

2. Materials and methods

2.1. Materials

Paclitaxel (PTX) was purchased from Hongdoushan Co. Ltd (Jiangsu, China). Cell Counting Kit-8 (CCK-8) was supplied by Dojindo Laboratories (Kumamoto, Japan). Coumarin-6 (Cou6) was bought from Sigma-Aldrich Chemical Corporation (St Louis, MO, USA). MDCK-MDR1 was purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Specific-pathogen free Sprague-Dawley (SD) rats were purchased from Yangzhou University Medical Centre (Yangzhou, China). All animal studies were performed in compliance with guidelines set by the Animal Care Committee at Drum Tower Hospital, Nanjing, China.

2.2. Preparations of paclitaxel loaded erythrocyte membrane nanoparticles (EMNP)

Erythrocyte membranes were prepared from natural red blood cells (RBCs) of SD rats by hypotonic hemolysis method [11]. Briefly, the blood obtained from rats was centrifuged at 1000×*g* for 8 min at 4 °C, and then the serum and the buffy coat were carefully removed. The resulting packed RBCs were washed in ice cold 1 × PBS for three times before hypotonic medium treatment. The washed RBCs were suspended in 0.25 × PBS in an ice bath for 40 min and were centrifuged at 10000 rpm for 5 min. The hemoglobin was removed, whereas the pink pellet was collected. 200 μL erythrocyte membrane (from 2 mL blood) were then resuspended in 1 mL cold isotonic PBS in a penicillin bottle. The erythrocyte membrane solution was treated by using bath sonicator for 5 min at a frequency of 37 KHz and power of 565 W, followed by adding 25 μL of 20 mg/mL PTX ethanol solution to sonicate another 5min. PTX loaded erythrocyte membrane nanoparticles (EMNP) were stored in 4 °C for further use.

2.3. Characterization of EMNP

The particle size and size distribution of EMNP were measured by the dynamic light scattering (DLS) method using a Brookhaven analyzer (Brookhaven Instruments Corporation). To visualize the structure of EMNP, the samples were dried on a copper grid coated with amorphous carbon, then negatively stained with 2% (w/v) phosphotungstic acid and observed through transmission electron microscopy (TEM, EM-200CX, JEOL, Japan).

To test the drug loading (DL) and encapsulation efficiency (EE) of EMNP, the EMNP solution was dissolved in acetonitrile to extract PTX, then the content of PTX was subsequently analyzed by HPLC. Acetonitrile/water (55/45, v/v) was used as the mobile phase at a flow rate of 1.0 mL per minute [15].

To characterize the *in vitro* release behavior of the PTX from EMNP, an equilibrium dialysis method was performed. EMNP (containing 0.5 mg PTX) was added into a sealed dialysis membrane bag (MW 9 kDa), and then the bag was incubated in 30 mL of release medium (PBS contaning0.5% Tween 80, pH7.4) at 37 °C with a stirring speed of 100 rpm. At predetermined time intervals, a 5 mL volume of release medium was withdrawn at 0, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, 9.0, 24.0, 48.0 and 72.0 h, following by a supplement of an equal volume of fresh release medium each time. The concentration of PTX in each sample was analyzed using HPLC mentioned above. The cumulative release percentage was calculated from the standard curve of PTX.

2.4. Cell viability and uptake of EMNP

MDCK-MDR1 cells isolated from canine distal renal tissue were used as a model of the human intestinal mucosa *in vitro* to determine the intestinal absorption of drugs. To evaluate the cytotoxicity of EMNP and free PTX on MDCK-MDR1 cell lines *in vitro* in the subsequent experiments, MDCK-MDR1 cells were seeded into a 96-well plate at a density of 5×10^3 cells per well and cultured at 37 °C in 5%CO_2 for 24 h.Then the cells were incubated with different concentrations of EMNP and free PTX (calculated by PTX). After 3 h of incubation, 10 μL of CCK-8 solution was added to each well followed by another 2 h of incubation. The absorbance of each well at 450 nm was measured by a microplate reader.

As PTX is not a fluorescence dye, we placed PTX by Coumarin-6 (Cou6) to prepare EMNP. MDCK-MDR1 cells (2×10^4/well) were seeded into glass bottom dishes (Sunnyvale, California, USA). After 24 h, the medium was replaced by the medium Cou6 loaded EMNP, or the free Cou6 (6.25 μg/mL, calculated by Cou6). After 2 h incubation, the cells were washed twice with PBS and fixed with 4% paraformaldehyde solution for 20 min, then washed with PBS, and stained with DAPI for another 15 min. The fixed cells were observed by confocal laser scanning microscope (Leica TCS SP5, GER). The excitation/emission wavelengths for Cou6 were 466/504 nm.

2.5. Permeation of EMNP through MDCK-MDR1 cell monolayers

To evaluate the transport efficiency of EMNP and free PTX across the cultured cell monolayer, MDCK-MDR1 cells were seeded onto Falcon 24-multiwell insert system at a density of 6×10^5 cells/mL.

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