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# Pharmacokinetics and Tissue Distribution of Folate-Decorated Human Serum Albumin Loaded With Nano-Hydroxycamptothecin for Tumor Targeting

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ABSTRACT

The goal of this work is to develop the method of preparing folate (FA)-decorated human serum albumin (HSA) loaded with nano-hydroxycamptothecin (nHCPT) nanoparticles (NPs) (FA-HSA-nHCPT-NPs) and to explore its antitumor activity *in vivo* and *in vitro*. FA-HSA-nHCPT-NPs were obtained by preparing nHCPT by a high-pressure homogenization technique followed with an anti-solvent method. The drug-loading efficiency of the FA-HSA-nHCPT-NPs was 7.8%. We adopted the human breast cancer cells (FA receptor-expressing MCF-7 cells) and BALB/c mice inoculated with human MCF-7 cells to determine the antitumor activity of FA-HSA-nHCPT-NPs *in vitro* and *in vivo*, respectively. The antitumor activity of FA-HSA-nHCPT-NPs was stronger than that of the raw HCPT in both conditions. Tissue distribution analysis showed that the FA-HSA-nHCPT-NPs carried more HCPT to tumors than the raw HCPT. The tumor inhibitory rate of FA-HSA-nHCPT-NPs was much higher compared with the raw HCPT. Th7us, the FA-HSA-nHCPT-NPs could serve as a viable delivery system with an obvious target effect on tumor.



## Introdution

Camptothecin (CPT) and related analogs, which could inhibit DNA replication and RNA transcription by forming a CPT-topoisomerase I-DNA triplet and result in cell death because of fractured DNA double helix structure,[1,2] are used as a chemotherapeutic agent for various cancers. However, CPT is not used clinically because of its poor solubility and high systematic toxicity.[3] Hydroxycamptothecin (HCPT), an natural derivative of CPT, has shown stronger antitumor effects with fewer side effects and less toxicity in animal and in human trials compared to CPT. Therefore, HCPT has been widely used in the treatment of various cancers, such as gastric carcinoma, hepatocarcinoma, leukemia, breast cancer, colon cancer, tumor of the head and neck, and human squamous cell carcinoma of the tongue.[4,5] Nevertheless, its poor solubility in water and physiological acceptable organic solvents have limited its practical use.[6] The E-ring's stability has also restricted its clinical application.[7] To solve these problems, modifying its formulation through emulsions, micelles, liposomes, and nanoparticles (NPs) have been explored with some success.[8–11] For example, some new types of NPs could retard the lactone ring opening *in vivo*.[2,12] Human serum albumin (HSA) is a multigene family of proteins in the circulation system with a mean molecular weight of 66 kDa and a blood concentration of 50 mg/mL.[13,14] Due to its low toxicity, high biocompatibility and biodegradation rate, the HSA has become an ideal carrier for anticancer agents.[15] In recent years, targeted drug delivery systems were widely studied because it could deliver more drugs to the target tissue than to the normal organs thus relieving the drug side effect.[16,17] Folate (FA), a type of Vitamin B, is involved in the biosynthesis of purines and

*Abbreviations used:* CPT, camptothecin; DMEM, Dulbecco's modified eagle medium; FA, folate; FA-HSA-nHCPT-NPs, folate-decorated human serum albumin loaded with nano-hydroxycamptothecin nanoparticles; HCPT, hydroxycamptothecin; HPLC, high-performance liquid chromatography; HSA, human serum albumin; nHCPT, nano-hydroxycamptothecin; NPs, nanoparticles; TPT, topotecan.

*Conflict of interest:* All the authors have approved the manuscript and agreed with submission in this journal. There are no conflicts of interest to declare.

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pyrimidines and is essential for DNA synthesis.[18,19] The FA receptor is frequently overexpressed in cancer cells in contrast to normal cells.[20,21] For these reasons, FA has become one of the most important targeting groups. In our study, FA-HSA-HCPT-NPs (folate-decorated human serum albumin loaded with nano-hydroxycamptothecin nanoparticles) were prepared, and we investigated the release curve, physical property, pharmacokinetics, and antitumor activity of FA-HSA-HCPT-NPs *in vitro* and *in vivo* using FA receptor-expressing MCF-7 cells.[22,23] The FA-HSA-HCPT-NPs displayed slow release, high tumor inhibitory rate, and outstanding tumor targeting properties.

## Materials and Methods

### *Materials*

HCPTs (98%) were provided by Hisun Pharmaceutical Company Ltd (Zhengjiang, China). HSA was purchased from Harbin Triple Pharmaceutical Company Ltd (Harbin, China). Topotecan (TPT) was purchased from Tianyuan Technology Company Ltd (Chengdu, China). Dulbecco's modified eagle medium (DMEM) was purchased from Gibco (Invitrogen, Carlsbad, CA). Folic acid, FA-free DMEM, phosphate-buffered solution (pH 7.4), and trypsin were purchased from Sigma-Aldrich (St. Louis, MO). The water used was pretreated with the Milli-Q plus system (Millipore, Bedford, MA). Acetonitrile and methanol were of high-pressure liquid chromatography grade and all the other reagents were of analytical grade.

### *Preparation of nHCPT*

The micro powder of the raw HCPT was prepared by an anti-solvent method to get the mean particle size under 1000 nm.[24] Briefly, 60 mg raw HCPT was dissolved in 1 mL dimethyl formamide, and then 2.5 mL chloroform was added dropwise to the HCPT solution under stirring. After 15 min, the mixture solution was centrifuged at 12,000 rpm for 10 min. The precipitate was processed to remove the dimethyl formamide and chloroform by supercritical $CO_2$ fluid extraction. The obtained HCPT micro powder was dispersed to deionized water, and a high-pressure homogenization technique was used to prepare nHCPT. The 3 essential parameters in high-pressure homogenizing process including the pressure of the homogenizer, the concentration of HCPT solution, and the numbers of cycles were optimized to 800 bar, 0.5 mg/mL, and 15 cycles, respectively.

### *Preparation of FA-HSA*

FA-HSA was prepared by the methods reported before.[25] Briefly, after 5 mg of *N*-hydroxysuccinimide ester of FA was dissolved in 1 mL carbonate-bicarbonate buffer (pH 10), this solution was added to 20 mL HSA solution (20 mg/mL) under stirring and kept reacting for another 90 min at room temperature. A total of 0.1 M hydrochloric acid was added dropwise to the mixed solution adjusting the pH to 4-5 to precipitate unreacted *N*-hydroxysuccinimide ester of FA. The mixture was stirred for 30 min and centrifuged at 10,000 rpm for 10 min for 3 times. The supernatant was lyophilized for subsequent usage.

### *Preparation of FA-HSA-nHCPT-NPs*

The method used for FA-HSA-nHCPT-NP preparation was reported in our previous study.[26] Briefly, 12 mg nHCPT was dispersed to 24 mL deionized water and sonicated for 10 min. The pH of the suspension was adjusted to 6.0 to keep nHCPT lactone formation by acetic acid. Then the suspension was added dropwise to the FA-HSA aqueous solution under stirring. The ethanol-water solution (4:1, vol/vol) was dripped into the mixture by a peristaltic pump and 1.2 mL 0.25% glutaraldehyde (wt/vol) was added dropwise to the suspension as a cross-linking agent. The reaction was stirred for 18 h at room temperature. The mixture was centrifuged at 40,000 rpm for 10 min. The precipitation was redissolved in ultrapure water. Mannitol was added as a lyoprotectant before the solution was lyophilized.

### *Drug-Loading Efficiency*

The enzymolysis technology was adopted to determine the drug-loading efficiency. The concentration of HCPT was examined by high-performance liquid chromatography (HPLC). Briefly, 3 mg FA-HSA-nHCPT-NPs was diluted with 3 mL trypsin-phosphate buffered saline (PBS) solution (pH 7.4) under string. A total of 10 μL solution was added to 990 μL dimethylsulfoxide after 36 h. The solution was mixed fully and centrifuged at 12,000 rpm for 10 min. Then the supernatant was injected into the HPLC system. A high-pressure liquid chromatography system equipped with a Jasco 975 ultraviolet detector and HIQ SIL C18 column (250 mm × 4.6 mm, 5 μL; Jasco, Tokyo, Japan) were used. The mobile phase consisted of acetonitrile and water (35:65, vol/vol) adjusted to pH 5.5 by acetic acid at the flow of 1 mL/min. The measuring wavelength was 370 nm. All samples were detected in triplicate and the amount of HCPT in the NP suspension was calculated. The drug-loading efficiency and encapsulation efficiency were calculated by the following formula:

$$\textit{Drug loading efficiency}\ (\%) = \textit{weight of the drug in nanoparticles} / \textit{weight of the nanoparticles}$$

### *Characterization of NPs*

#### *Particle Size and Surface Charge*

The particle size and surface potential of nHCPT and FA-HSA-nHCPT-NPs were detected by laser particle analyzer and zeta potential analyzer, respectively. nHCPT and FA-HSA-nHCPT-NPs were dispersed into deionized water and diluted to appropriate concentration. Both particle size and zeta potential measurements were determined in triplicate and the results were the average of 3 measurements.

#### *Physical Status of HCPT and FA-HSA-nHCPT-NPs*

The surface morphology of FA-HSA-nHCPT-NPs was observed by scanning electron microscopy (FEI Company, Eindhoven, The Netherlands). An X-ray diffractometer (Philips, Xpert-Pro, The Netherlands) was used to detected the physical status of raw HCPT, nHCPT, and FA-HSA-nHCPT-NPs. The scanning rate was 5°C/min, and the diffraction angle ($2\theta$) was recorded at 5°C-80°C. Cu $K\alpha_1$ radiation was generated at 30 mA and 50 kV as the X-ray source.

### *HCPT Release From FA-HSA-nHCPT-NPs* In Vitro

*In vitro* release of HCPT from the FA-HSA-nHCPT-NPs was studied by membrane dialysis against PBS (pH 7.4). Briefly, 1 mL of FA-HSA-nHCPT-NPs (1 mg HCPT/mL) solution was placed in a dialysis bag (molecular weight cutoff 3500 Da). The entire system was immersed in 100 mL PBS solution at 37°C with stirring. At designated time intervals, 15 mL of release medium was removed and replaced with the same volume of fresh PBS solution. The concentration of HCPT in the release medium was detected by HPLC