Human Serum Albumin-grafted Cationic Solid Lipid Nanoparticles for Delivery of Nevirapine in Vivo

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Abstract—Body distribution of cationic solid lipid nanoparticles (CSLNs) grafted with human serum albumin (HSA) was analyzed by conjugation of octadecylamine-fluorescein isothiocyanate (O-F) and intravenous administration to mice. The fluorescent images indicated that the quantity of HSA-grafted O-F-CSLNs, internalized inside brain, heart, liver, kidney, spleen, and lung, was larger than that of HSA-free O-F-CSLNs. The uptake of HSA-grafted O-F-CSLNs in the brain was about three times that of O-F-CSLNs.

Keywords—body distribution; human serum albumin; cationic solid lipid nanoparticle; nevirapine; delivery

I. INTRODUCTION

The delivery of lipid pellets via oral administration was developed in the early 1990s [1]. Solid lipid nanoparticles (SLNs) with the advantages of polymer, lipid, and liposome significantly improved the characteristics of lipid pellets [2,3]. Hence, SLNs could be suitable for intravenous (i.v.) injection [4]. In addition, SLNs extended drug circulation in rodents and promoted the delivery of doxorubicin and FUDR to the brain [5,6]. The transport of SLNs to the lymph and blood plasma in rats was observed by photon correlation spectroscopy [7]. Moreover, SLNs could increase the uptake of camptothecin in the reticuloendothelial system (RES) and prolonged the drug residence in the brain and heart [8].

The aim of this study is to investigate the body distribution of NVP using human serum albumin (HSA)-grafted cationic SLNs (CSLNs). Fluorescent octadecylamine-fluorescein isothiocyanate (FITC)-conjugated CSLNs (O-F-CSLNs) were used to analyze the brain-targeting effect in mice.

II. EXPERIMENT

A. Reagents and Chemicals

FITC isomer and formaldehyde solution were purchased from Sigma (St. Louis, MO). Octadecylamine was obtained from Fluka Biochemika (Buchs, Switzerland). N-Dimethylformamide was purchased from J. T. Baker (Phillipsburg, NJ). Ketamine was obtained from Nang Kuang (Tainan, Taiwan). Tissue-tek optimal cutting temperature compound (TOCTC) was purchased from Sakura Finetek (Torrance, CA). Aqua mounter was obtained from Bio SB (Santa Barbara, CA). Nevirapine (NVP) was purchased from ChemPacific (Baltimore, MD).

B. Preparation of O-F-CSLNs and HSA/O-F-CSLNs

CSLNs were fabricated by the method described previously [9]. Fluorescent CSLNs were prepared by encapsulating octadecylamine-FITC (O-F) in NVP-CSLNs and HSA/NVP-CSLNs [10]. 0.01% (w/v) octadecylamine and 0.03% (w/v) FITC were dissolved in N, N-dimethylformamide and reacted at 50°C for 48 h. O-F was precipitated out by adding ultrapure water. After wash with ultrapure water, the O-F sediment was rehydrated by adding ultrapure water. After wash with ultrapure water, the O-F sediment was rehydrated by adding ultrapure water. After wash with ultrapure water, the O-F sediment was rehydrated by adding ultrapure water. After wash with ultrapure water, the O-F sediment was rehydrated by adding ultrapure water. After wash with ultrapure water, the O-F sediment was rehydrated by adding ultrapure water. After wash with ultrapure water, the O-F sediment was rehydrated by adding ultrapure water. After wash with ultrapure water, the O-F sediment was rehydrated by adding ultrapure water.

C. Body Distribution of HSA/O-F-CSLNs and O-F-CSLNs

Male mice of Institute of Cancer Research with body weight of 25–35 g were purchased from BioLASCO (Taipei, Taiwan) and housed in Laboratory Animal Center of Chang Gung Memorial Hospital in Chia-Yi according to the institutional guidelines. The animal experiment protocol was approved by the Institutional Animal Care and Use Committee of Chang Gung Memorial Hospital in Chia-Yi. The mice were maintained on hardwood pads in temperature-controlled surroundings with a 12-h light-dark cycle and fasted for 12 h before i.v. administration. After fixation, the suspensions containing HSA/O-F-CSLNs or O-F-CSLNs were administered by i.v. injection via tail vein. The dose was 66 mg/kg. The weight percentage of CA in CA and SA was 50% and the concentration of HSA was 0.5 mg/mL. After 30 min, the mice were sacrificed via intraperitoneal injection with ketamine. The dose of anaesthetic was 1.5 mL/kg. The sacrificed mice were operated for bloodletting via arteria coeliaca before surgical dissection. The brain, heart, liver, kidney, spleen and lung were taken out, cleaned, frozen on...
dry ice for 5 min and stored at -80°C. Fig. 1 shows the typical frozen organs. The frozen organs were embedded in TOCTC, sliced by a cryostat microtome (CM3050-S, Leica, Wetzlar, Germany) into sections of 0.16 µm at -20°C, fixed in 10% (v/v) formaldehyde solution for 10 min and sealed with aqua mounter. The optical and fluorescent images were obtained by an inverted microscope (Eclipse TS-1000-F, Nikon, Tokyo, Japan) and by a fluoromicroscope (E600, Nikon, Tokyo, Japan) at green wavelength of 488 nm, respectively. The distribution of HSA/O-F-CSLNs and O-F-CSLNs in the brain was viewed by a confocal laser scanning microscope (Axioskop 2 plus, Zeiss, Oberkochen, Germany) at 488 nm. The fluorescence intensity was analyzed by Image-Pro Plus (version 4.5, Media Cybernetics, Bethesda, MD) with the green fluorescence between 100 and 180 in the red-green-blue color model.

III. RESULTS AND DISCUSSION

A. Body Distribution of Fluorescent Nanocarriers

Fig. 2 shows the typical fluorescent distribution of HSA/O-F-CSLNs and O-F-CSLNs in the brain, heart, liver, kidney, spleen, and lung of mice after i.v. administration. As exhibited in these images, the two carriers could be internalized inside the organs. This was mainly because the surface properties and opsonization of the injected lipid formulations led to the tissue uptake. In addition, Fig. 2 suggested that these lipid colloids were accumulated in the RES organs and could release NVP in the tissues at a slow rate [11,12]. It has been concluded that adherent opsonins on the particle surface could be recognized by the RES [13]. It has also been observed that after i.v. administration to rats, lipid emulsions could be absorbed by the RES in the liver.
Figure 2. Distribution of lipid particles in organs. (a-1)-(m-1): HSA/O-F-CSLNs; (a-2)-(m-2): O-F-CSLNs. (a-1), (b-1), (a-2) and (b-2): brain; (c-1), (d-1), (c-2) and (d-2): heart; (e-1), (f-1), (e-2) and (f-2): liver; (g-1), (h-1), (g-2) and (h-2): kidney; (i-1), (j-1), (i-2) and (j-2): spleen; (k-1), (l-1), (k-2) and (l-2): lung. (a-1), (c-1), (g-1), (l-1), (a-2), (c-2), (g-2) and (l-2): optical images; (b-1), (d-1), (f-1), (h-1), (j-1), (m-1), (b-2), (d-2), (f-2), (h-2), (j-2) and (m-2): fluorescent images.

and spleen [14]. Furthermore, as revealed in Fig. 2, HSA/O-F-CSLNs yielded a stronger fluorescent intensity than O-F-CSLNs, in general. The enhanced fluorescent intensity of HSA/O-F-CSLNs was due to the high affinity of HSA to the tissues.
Figure 3. Fluorescence in the brain under confocal laser scanning microscope. (a) HSA/O-F-CSLNs; (b) O-F-CSLNs.

B. Brain Image by Confocal Laser Scanning Microscope

Fig. 3 shows the fluorescent images of the brain tissue with the uptake of HSA/O-F-CSLNs and O-F-CSLNs under confocal laser scanning microscope. As indicated in this figure, the two lipid particles could penetrate into the brain, a non-RES organ, to a certain extent. It has been observed that after i.v. administration to rabbits, SLNs could increase the concentration of doxorubicin in the brain [15]. The present formulation using Tween 80 also enhanced the transport of CSLNs into the brain. It has been demonstrated that Tween 80 modified on drug-loaded particulate carriers could improve the permeability across the blood-brain barrier (BBB) after i.v. administration [16]. Moreover, based on the Image-Pro Plus analysis, the fluorescent intensity in Fig. 3 (a) was about three times (591.9/197.9) that in Fig. 3 (b). This indicated that HSA/O-F-CSLNs were easier to cross the BBB than O-F-CSLNs. This might result from the binding of HSA with apolipoprotein E3, A-I or B-100 to promote the transport into the central nervous system [17]. Therefore, HSA on the particle surface could improve the permeability of CSLNs and therapeutic efficacy of NVP for the treatment of human immunodeficiency virus residing in the brain.

IV. CONCLUSIONS

After i.v. administration to mice, HSA/O-F-CSLNs yielded a higher fluorescent intensity than O-F-CSLNs in brain, heart, liver, kidney, spleen, and lung. The fluorescent intensity of HSA/O-F-CSLNs in the brain was about three times that of O-F-CSLNs, indicating that the grafted HSA favored the transport of CSLNs across the BBB.

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NOMENCLATURE

BBB: blood-brain barrier
CSLN: cationic solid lipid nanoparticle
FITC: fluorescein isothiocyanate
HSA: human serum albumin
HSA/O-F-CSLN: octadecylamine-FITC-conjugated NVP-CSLN with surface HSA
NVP: nevirapine
O-F-CSLN: octadecylamine-FITC-conjugated NVP-CSLN

REFERENCES


