Effect of the amount of cationic lipid used to complex siRNA on the cytotoxicity and proinflammatory activity of siRNA-solid lipid nanoparticles

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Pharmacokinetics and biodistribution of siRNA-SLNs prepared at the N/P ratio of 12:1

1. Materials and Methods

1.1. Pharmacokinetics of siRNA-SLNs prepared at the N/P ratio of 12:1

The animal protocol was approved by the Institutional Animal Care and Use Committee at The University of Texas at Austin. C57BL/6 mice were from Charles River Laboratories (Wilmington, MA). To evaluate the plasma and tissue pharmacokinetics (PK) of the siRNA-SLNs prepared at the N/P ratio of 12:1, 18 female C57BL/6 mice were intravenously injected with siRNA-SLNs labeled with TopFluor® cholesterol. Three more mice were injected with sterile PBS as a control. At predetermined time points (i.e., 0.5, 2, 4, 12, 24, 48 h), mice (n = 3) were euthanized to collect blood samples and key organs. Fluorescence intensity in the samples was measured using a microplate reader (blood) or a Spectrum IVIS imaging system (Perkin Elmer, Chicago, IL) at the excitation and emission wavelengths of 465 nm and 500 nm, respectively (organs). Plasma PK parameters were derived using PK Solver software (Zhang et al., 2010), assuming a single dose two-compartmental model.

1.2. Biodistribution of siRNA-SLNs prepared at N/P ratios of 34:1 and 12:1

Nine healthy female mice (20-23 g) were randomized into 3 groups to receive intravenous injection of siRNA-SLNs prepared at N/P ratios of 34:1 or 12:1, or 10 mM PBS as a control. The dose of siRNA was 0.5 mg/kg, and the SLNs were labeled with TopFluor® cholesterol. Twelve hours later, mice were euthanized, key organs including pancreas, spleen, kidneys, liver, heart, lung, ovaries and uterus, brain, stomach, small and large intestine were harvested, washed with PBS, and then imaged with a Spectrum IVIS imaging system. Blood was collected by cardiac puncture into heparinized tubes, and the fluorescence intensity of the blood was measured using a microplate reader. Data were normalized by the weight of the organs or the volume of the blood. It is noted that the PK and biodistribution studies were done by measuring fluorescently labeled cholesterol in the SLNs. In vitro release data confirmed that the release of the TopFluor cholesterol from the siRNA-SLNs was minimal (e.g., less than 1% in 6 h in PBS (10 mM, pH 7.4) with 10% (v/v) of mouse serum).

2. Results and Discussion

Because the siRNA-SLNs prepared at the N/P ratio of 12:1 (N/P, 12:1) were less cytotoxic than siRNA-SLNs prepared at other N/P ratios and were not more proinflammatory, the PK and distribution profiles of them were investigated in a healthy mouse model (Fig. S1). The plasma PK of the siRNA-SLNs followed a standard two-phase profile with a rapid clearance within the first 8 h, followed by a slower elimination with a calculated $t_{1/2}$ value of about 70 h (Fig. S1A, Table S1). Table S1 also contains other selected PK parameters. The volume of distribution (V_d) of the siRNA-SLNs was much larger than the volume of the mouse body fluid (i.e., ~ about 2 mL), indicating that siRNA-SLNs accumulated significantly in the organs, as shown in Fig. S1B. As to the tissue/organ PK profiles, the siRNA-SLNs accumulated relatively more in the lung and kidneys, with a t_{max} at 24 h, whereas in the other organs (spleen, liver, heart), the t_{max} was 12 h (Fig. S1B).

In addition, the biodistribution of the siRNA-SLNs (N/P, 12:1) in a mouse model was evaluated and compared to that of siRNA-SLNs (N/P, 34:1). Data in Fig. S2 show that the distribution of the siRNA-SLNs (N/P, 12:1) in all major organs evaluated was higher than the siRNA-SLNs (N/P, 34:1), likely because the siRNA-SLNs (N/P, 12:1) circulated longer in the blood than the siRNA-SLNs (N/P, 34:1) (Fig. 2A, inset). The siRNA-SLNs (N/P, 12:1) had a negative zeta potential and a smaller particle size, while the siRNA-SLNs (N/P, 34:1) had a positive zeta potential and relative larger particle size, which may have contributed to their different biodistribution profiles. Indeed, there are reports supporting the effect of particle size and surface charge on the biodistribution of nanoparticles in different organs after intravenous administration (Chouly et al., 1996; Kulkarni and Feng, 2013; Sonavane et al., 2008).

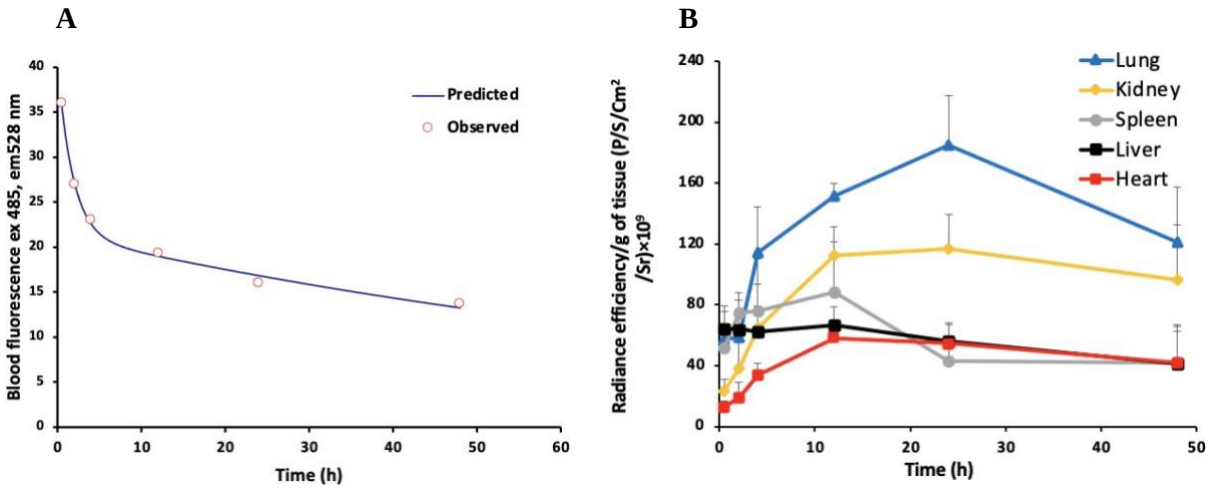

Figure S1. Concentration vs. time profiles of siRNA-SLNs in mouse blood (A) and key organs (B). Mice were i.v. injected with 0.5 mg of siRNA per kg body weight in siRNA-SLNs prepared at the N/P ratio of 12:1. Shown in A is fluorescence intensity per 100 µL of blood, and in B is fluorescence intensity per g of tissue. Data are mean ± S.D. (n = 3).

Table S1. Blood PK parameters of siRNA-SLNs prepared at the N/P ratio 12:1.

PK Parameter	Unit	Value
Elimination half-life time ($t_{½\beta}$)	h	69.5
Volume of distribution (V_d)	ml	12.3
Clearance (Cl)	ml/h	0.23
Area under the curve $_{0\text{-}t}$ ($AUC_{0\text{-}t}$)	(Fluorescence intensity unit/0.1 ml) h	851.2
Area under the curve $_{0\text{-infinity}}$ ($AUC_{0\text{-inf}}$)	(Fluorescence intensity unit/0.1 ml) h	2182.3

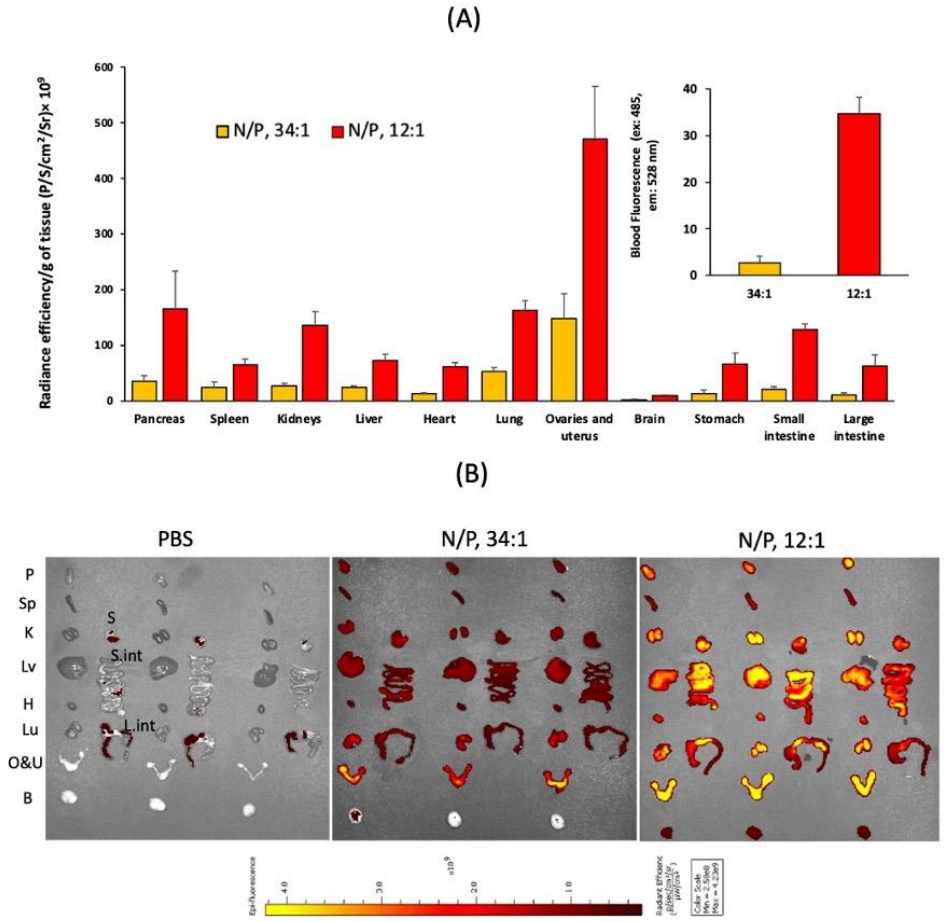

Figure S2. The biodistribution profiles of siRNA-SLNs prepared at N/P ratios of 12:1 or 34:1. (A) Florescence intensity per g in each organ or per 0.1 mL of blood (inset). (B) IVIS imaging of organs (P = Pancreas, Sp = Spleen, K = kidneys, Lv = Liver, H = Heart, L = Lung, O&U = Ovaries and Uterus, B = Brain, S = Stomach, S.int = Small Intestine, L.int = Large Intestine).

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