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Physicochemical and pharmacokinetic characteristics of cationic liposomes

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After intravenous administration of plasmid DNA (pDNA)/cationic liposome complexes, the gene expression is predominantly observed in the lung due to the physicochemical properties of the liposome complexes and the physiology of the lung. To determine the physicochemical properties and distribution behavior of cationic liposomes for lung-selective drug and/or gene delivery systems, *N*-[1-(2,3-dioleyloxy)propyl]-*n*,*n*,*n*-trimethylammonium chloride (DOTMA)/cholesterol and 1,2-dioleoyl-3-trimethylammoniopropane (DOTAP)/cholesterol liposomes were studied. The particle sizes of DOTMA/cholesterol and DOTAP/cholesterol liposomes were very similar: 126 and 128 nm, respectively. Furthermore, the zeta potentials of these two liposomes were 51 and 66 mV, respectively. After intravenous injection into mice, both cationic liposomes were rapidly eliminated from the blood circulation and preferentially recovered in the lung. Interestingly, the highest lung accumulation was observed at 1 min, and then, decreased gradually. The distribution characteristics of DOTMA/cholesterol and DOTAP/cholesterol liposomes were almost identical due to the similarities in their physicochemical properties. These results demonstrated that DOTMA/cholesterol and DOTAP/cholesterol liposomes, which possess a positive charge, are promising carriers for lung-selective drug and/or gene delivery systems.

1. Introduction

Recently, much effort has been devoted to the development of cationic liposome-mediated gene delivery systems due to their favorable characteristics. Cationic liposomes condense plasmid DNA (pDNA) into particulates of defined size, protect them from premature degradation in the bloodstream, and interact non-specifically with cell surfaces (Mahato et al. 1997; Huang and Li 1997; Kawakami et al. 2002; Ruozi et al. 2003). Since pDNA complexed with cationic liposomes (i.e. lipoplexes) accumulate in the lung immediately after intravenous administration (Mahato et al. 1995; Sakurai et al. 2001), they lead to a high gene expression in the lung (Zhu et al. 1993; Li and Huang 1997; Song et al. 1997; Kawakami et al. 2000a). Although lung-selective drug and/or gene delivery by cationic liposomes is also expected, there are few reports on the distribution of (bare) cationic liposomes after intravenous administration.

The purpose of this study was to elucidate the physicochemical and distribution characteristics of cationic liposomes for lung-selective delivery systems. *N*-[1-(2,3-dioleyloxy)propyl]-*n*,*n*,*n*-trimethylammonium chloride (DOTMA)/cholesterol and 1,2-dioleoyl-3-trimethylammoniopropane (DOTAP)/cholesterol liposomes were selected as cationic liposomes because of the many reports regarding their *in vivo* gene transfection in the lung after intravenous administration (Song et al. 1997; Li and Huang 1997; Templeton et al. 1997; Mahato et al. 1998; Kawakami et al. 2000a; Sakurai et al. 2001; Faneca et al. 2004). [^{3}H]Cholesteryl hexadecyl ether (CHE), a non-degradable maker, was employed as a liposomal marker (Schwiegelshohn et al. 1995; Hattori et al. 2000; Kawakami et al. 2000b; Managit et al. 2003).

2. Investigations, results and discussion

Liposomes are widely used as carriers for a variety of drugs. After intravenous administration, however, they are commonly retained in the blood circulation and removed by the reticuloendothelial system (Papahadjopoulos and Gabizon 1990). Therefore, tissue and/or cell-specific drug delivery is of great importance for a variety of clinical purposes. In order to achieve tissue and/or cell-specific drug delivery, the liposomal surface can be modified with a variety of agents including polyethyleneglycol (Klibanov et al. 1990; Teshima et al. 2004), galactose (Kawakami et al. 2001; Mady et al. 2004), mannose (Kawakami et al. 2000b; Engel et al. 2003), transferrin (Ishida et al. 2001), antibody (Zhang et al. 2003; Yu et al. 2004), RGD-peptide (Dubey et al. 2004), and folate (Pan et al. 2003). In this present study, we analyzed the disposition characteristics of cationic liposomes for tissue and/or cell-specific drug delivery.

Table 1 summarizes the zeta potentials and mean particle sizes of cationic liposomes. The zeta potentials of DOTMA/cholesterol and DOTAP/cholesterol liposomes were

Table 1: Mean particle sizes and zeta potentials of cationic liposomes

Cationic liposomes	Mean particle size (nm)	Zeta potential (mV)
DOTMA/cholesterol liposome	125.5 ± 4.1	51.2 ± 2.3
DOTAP/cholesterol liposome	127.6 ± 7.9	66.5 ± 1.7

Each value represents the mean ± S.D. values (n = 3)

51.2 and 66.5 mV, respectively. As shown by the zeta potential, the surface charge of each particle was positive. The mean particle sizes of DOTMA/cholesterol and DOTAP/cholesterol liposomes were 126 and 128 nm, respectively. Therefore, there was no significant difference in physicochemical properties between DOTMA/cholesterol and DOTAP/cholesterol liposomes.

After intravenous administration of [^{3}H]CHE labeled DOTMA/cholesterol and DOTAP/cholesterol liposomes, they were rapidly eliminated from blood circulation (Fig. 1). The highest amount of lung accumulation of both liposomes reached about 280% of dose/g of tissue at 1 min and then decreased gradually. Their concentrations in the liver and spleen were relatively low; i.e., about 40–50% and 35% of dose/g of tissue, respectively (Fig. 2).

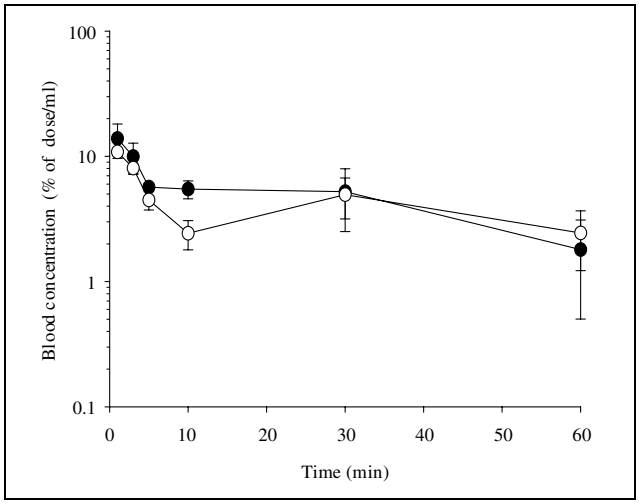

Fig. 1: Blood concentration of [^{3}H]CHE-labeled DOTMA/cholesterol (●) and DOTAP/cholesterol (○) liposomes following intravenous injection in mice. Each value represents the mean ± S.D. (n = 3)

Once the [^{3}H]CHE labeled liposomes were taken up by the organ, they began to redistribute. To avoid this complication, pharmacokinetic analysis was performed during a relatively early period (10 min) when the tissue uptake efflux was negligible. Table 2 summarizes the AUC, and tissue uptake index for representative organs after intravenous administration of cationic liposomes. This pharmacokinetic analysis revealed that the disposition of cationic liposomes was characterized by a small AUC. The tissue uptake index for the lung was the greatest among the tissues examined. The lung uptake index of DOTMA/cholesterol was 130000 µl/h/g, thus, the figure for DOTAP/cholesterol liposomes was around 220000 µl/h/g which was quite closed to the pulmonary plasma flow rate 260000 µl/h/g (Gerlowski and Jain 1983). There was a slight difference in pharmacokinetics between DOTMA/cholesterol and DOTAP/cholesterol liposomes, however, these cationic liposomes were predominantly accumulated in the lung after intravenous administration.

As for the other cationic liposome systems, Litzinger et al. (1996) investigated the biodistribution characteristics of the cationic liposomes 3β-(*N*-(*N*′,*N*′-dimethylaminoethane)carbamoyl)cholesterol (DC-Chol) and dioleoylphosphatidylethanolamine (DOPE) liposomes. After intravenous injection into the tail vein of mice (at 5 min) DC-Chol/DOPE liposomes accumulated extensively in the liver (about 75% of the dose), whereas less accumulated in the lung (about 10% of the dose). The distribution differences between DC-Chol/DOPE liposomes and DOTMA/cholesterol liposomes (Fig. 2A) correspond to the observation involving gene delivery systems that there is lower gene expression in the lung after intravenous administration of pDNA complexed with DC-Chol/DOPE liposomes (Kawakami et al. 2000c) compared to pDNA complexed with DOTMA/cholesterol liposomes (Kawakami et al. 2000a).

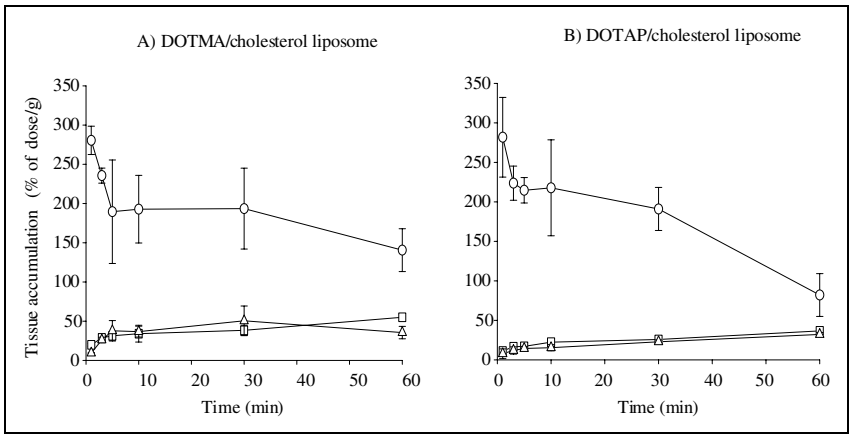

Fig. 2:
Tissue accumulation of [^{3}H]CHE-labeled DOTMA/cholesterol (A) and DOTAP/cholesterol (B) liposomes following intravenous injection in mice. Radioactivity was determined in the lung (○), liver (□) and spleen (△). Each value represents the mean ± SD (n = 3)

Table 2: Area under the blood concentration-time curve (AUC) and tissue uptake index of [^{3}H]CHE-labeled liposomes after intravenous injection into mice[a]

Cationic liposomes	AUC[a]	Tissue uptake index (µl/h/g)[a]		
	(% of dose · h/ml)	CL_{lung}	CL_{liver}	CL_{spleen}
DOTMA/cholesterol liposome	1.40	131218	27387	16947
DOTAP/cholesterol liposome	1.00	216178	22193	15260

[a] The AUC and tissue uptake index were calculated for the periods up to 10 min after injection. An average of three experiments is shown

We previously analyzed the physicochemical and disposition characteristics of pDNA complexed with DOTMA/cholesterol liposomes (Sakurai et al. 2001). When pDNA was complexed with DOTMA/cholesterol liposomes at a charge ratio of 2.24, the zeta potential and particle size were 46 mV and 187 nm, respectively. In this study, the zeta potential of DOTMA/cholesterol liposomes (about 51 mV) was higher than that of pDNA complexed with DOTMA/cholesterol liposomes (about 46 mV), suggesting neutralization by negatively charged pDNA. Although the particle size of DOTMA/cholesterol liposomes was almost the same between these studies, the particle size of DOTMA/cholesterol liposomes (about 126 nm) was smaller than that of pDNA complexed with DOTMA/cholesterol liposomes (about 187 nm), suggesting an interaction with negatively charged pDNA.

In our previous study, about 80% of [^{32}P]pDNA complexed with DOTMA/cholesterol liposomes had accumulated in the lung 1 min after injection (data not shown). A high degree of accumulation of lipoplexes would explain the gene expression level in the lung although it remains unclear why lipoplexes accumulate in the lung. Some studies have suggested that lipoplexes aggregate with blood components via electrostatic interaction and become entrapped in the lung capillaries (McLean et al. 1997; Sakurai et al. 2001). Although the zeta potential of DOTMA/cholesterol liposomes was higher than that of pDNA complexed with DOTMA/cholesterol liposomes, only 50% of the dose (the lung weight was about 0.2 g) of DOTMA/cholesterol liposomes accumulated in the lung 1 min after intravenous injection (Fig. 2). This difference in lung accumulation may be explained by the fact that the particle size of DOTMA/cholesterol liposomes was smaller than that of pDNA complexed with DOTMA/cholesterol liposomes.

Recently, Tan et al. (2001) reported that when pDNA was injected into the tail vein of mice 2–5 min after the injection of cationic liposomes (DOTAP/cholesterol liposomes), 50–80% lower levels of proinflammatory cytokines, including TNF-α, IL-12, and IFN-γ, were observed compared with lipoplex administration. Moreover, the sequential injection technique resulted in a 2- to 5-fold higher level of transgene expression in the lung while transgene expression at 10 or 20 min sequential injection intervals was at a level comparable with that of lipoplex administration. As far as sequential injection-mediated gene transfection is concerned, the distribution characteristics of cationic liposomes need to be investigated to clarify their mechanism. As shown in Fig. 2, the lung accumulation of DOTAP/cholesterol liposomes decreased gradually from 1 to 5 min. Subsequently, the radioactivity in the lung fell gradually from 5 to 30 min, suggesting a lower interaction with negatively charged endogenous components in the blood. Thus, these results may support the fact that an effective interval for sequential injection is within 5 min after administration.

In conclusion, we have demonstrated that DOTMA/cholesterol and DOTAP/cholesterol liposomes, which posses a positive charge, accumulate efficiently in the lung after intravenous administration. This observation provides valuable information to help in the design of cationic liposomes for drug and gene delivery.

3. Experimental

3.1. Materials

DOTMA were obtained from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). DOTAP was purchased from Avanti Polar Lipids Inc. (Alabaster, AL, USA). Cholesterol and Clear-Sol I were obtained from Nacalai Tesque, Inc. (Kyoto, Japan), and Soluene-350 was purchased from Packard Instrument Co., Inc. (Groningen, Netherlands). [^{3}H] CHE was purchased from NEN Life Science Products, Inc. (Boston, MA, USA). All other chemicals were of the highest purity available.

3.2. Preparation of liposomes

The preparation of liposomes was reported previously (Kawakami et al. 2000b, 2004). Briefly, a mixture of DOTMA or DOTAP and cholesterol was dissolved in chloroform and evaporated to dryness in a round-bottomed flask. Then, the lipid film formed was resuspended in 5 ml 5% dextrose solution. After hydration, the dispersion was sonicated for 5 min (200 W). Each resulting suspension was passed five times through a 0.45 μm pore size polycarbonate membrane filter (Millipore Co., Bedford, MA, USA). The concentration of liposomes was adjusted to 2.5 mg/ml total lipids based on radioactivity measurement. Radiolabeled liposomes were prepared by addition of [^{3}H]CHE (50 μCi) to the lipid mixture before formation of a thin film layer.

3.3. Measurement of particle sizes and zeta potentials

The particle sizes and zeta potentials of cationic liposomes without radioisotope were measured by laser-Doppler electrophoresis and dynamic light-scattering spectrophotometric methods using a Zetasizer Nano ZS (Malvern Instruments Ltd., Worcestershire, UK).

3.4. Distribution study

Five-week-old female ICR mice (20–30 g) were obtained from Shizuoka Agricultural Co-operative Association for Laboratory Animals (Shizuoka, Japan). All animal experiments were carried out in accordance with the Principles of Laboratory Animal Care as adopted and promulgated by the US National Institutes of Health and the Guideline for Animal Experiments of Kyoto University. [^{3}H]CHE (1.0 μCi/100 μl)-labeled liposomes were injected into the tail vein of mice at a dose of 25 mg/kg. At predetermined time points, blood was collected from the vena cava under anesthesia and the mice were then sacrificed. The liver, spleen, and lung were collected, washed with saline, blotted dry, and weighed. Ten microliters of blood and a precisely weighed small amount of each tissue (20–30 mg) were digested in 0.7 ml Soluene-350 by incubating overnight at 45 °C. Following digestion, 0.2 ml isopropanol, 0.2 ml 30% hydrogen peroxide, 0.1 ml 5 N HCl, and 5.0 ml Clear-Sol I were added. The samples were stored overnight and the radioactivity was measured in a liquid scintillation counter (LSC 6100, Aloka, Tokyo, Japan).

3.5. Calculation of tissue uptake index

Tissue distribution data were evaluated using the organ clearances as reported previously (Takakura et al. 1990; Kawakami et al. 2000b; Ishida et al. 2004). Briefly, the tissue uptake rate can be described by the following equation:

$$\frac{dX_t}{dt} = CL_{uptake} \cdot C_b \quad (1)$$

where X_t is the amount of [^{3}H]-labeled liposomes in the tissue at time t, CL_{uptake} is the tissue uptake clearance, and C_b is the blood concentration of [^{3}H]CHE-labeled liposomes. Integration of Eq. (1) gives

$$Xt = CL_{uptake} \cdot AUC_{(0-t)} \quad (2)$$

where $AUC_{(0-t)}$ represents the area under the blood concentration-time curve from time 0 to t. Eq. (2) divided by C_b gives

$$\frac{X_t}{C_b} = \frac{CL_{uptake} \cdot AUC_{(0-t)}}{C_b} \quad (3)$$

The CL_{uptake} value can be obtained from the initial slope of a plot of X_t/C_b vs. $AUC_{(0-t)}/C_b$.

The tissue uptake index was described as the uptake clearance per gram of tissue examined (μl/h/g).

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