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Relationship between particle size and lung retention time of intact solid lipid nanoparticle suspensions after pulmonary delivery

Zhengwei Huang [a], Ying Huang [a,b], Wenhua Wang [a], Fangqin Fu [b], Wenhao Wang [a], Shishuai Dang [a], Cheng Li [a], Cheng Ma [a], Xuejuan Zhang [c], Ziyu Zhao [d], Xin Pan [a,*], Chuanbin Wu [a,b]

[a] School of Pharmaceutical Sciences, Sun Yat-Sen University, Guangzhou, Guangdong 510006, PR China
[b] College of Pharmacy, Jinan University, Guangzhou 510632, PR China
[c] Institute for Biomedical and Pharmaceutical Sciences, Guangdong University of Technology, Guangzhou 510006, PR China
[d] Department of Pharmacology, Zhongshan School of Medicine, Sun Yat-Sen University, Guangzhou, Guangdong 510080, PR China

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ABSTRACT

The relationship between the particle size and lung retention time of inhaled nanocarriers was unclear, and this uncertainty hampered the design of nanocarriers for pulmonary delivery. The debate resulted from a lack of knowledge regarding the integrity of the involved nanocarriers. A distinguishable bioimaging probe which could differentiate between integrated and disintegrated nanocarriers by emitting different signals was introduced to address this problem. The aza-BODIPY structured aggregation-caused quenching (ACQ) probes were promising candidates, because they showed intense fluorescence signals in intact nanocarriers while quenched after the decomposition of nanocarriers. This attribute was called an on-off switch. In this paper, ACQ probes were encapsulated into a solid lipid nanoparticle suspension (SLNS) with different particle sizes (120–480 nm), and the relationship between particle size and lung retention time after pulmonary delivery was investigated in BALB/c mice. The results showed that a larger particle size led to a longer lung retention time. By comparing with the results of a non-water-quenching probe, the SLNS systems were found to be mostly intact in the pulmonary region. These findings will serve as a firm basis for the design and development of nanocarriers for pulmonary delivery.

1. Introduction

Carrier materials that are of nanometer size, viz. nanocarriers (NC), have been established as versatile vehicles for the delivery of drugs, biomacromolecules and vaccines via various administration routes, e.g. oral [1], intravenous [2], transdermal [3], ocular [4], pulmonary delivery [5], etc. The application of NC for pulmonary therapeutic delivery has been an area of intense active research in the nanomedicine community. This interest is owing to the increasing morbidity and mortality of pulmonary diseases, especially lung cancers. In 2019, pulmonary diseases are estimated to account for 142,670 deaths (nearly 25% of total cancer deaths) in the US [6]. A significant advantage of pulmonary-administered NC is that they can directly deliver the encapsulated payload to the lung region, which increases the local concentration whilst reducing extrapulmonary side effects [7]. Noticeably, a desirable attribute of these NC is a prolonged lung retention time when used as a localized delivery approach [8]; in this way, the drug-lesion interplay and therapeutic effect can be strengthened.

However, the use of NC for pulmonary delivery has not yet transitioned from bench to bedside. A vital obstacle is the limited knowledge of the correlation between the physicochemical properties and the in-vivo fate of inhaled NC [9]. Many studies have investigated the design of NC with different physicochemical characteristics [10], but the studies of in-vivo fate lag far behind. One of the crucial issues pending addressed regarding the in-vivo fate is the relationship between the NC physicochemical characteristics and the lung retention time.

As size control is one of the most feasible and reliable approaches by which the physicochemical characteristics of NC can be modified, efforts have been made to prolong their lung retention time by tuning the particle size. However, controversy remains about the relationship between particle size and lung retention time. Proportional correlation between particle size and lung retention time has been reported by

* Corresponding authors.
E-mail addresses: hzhengw3@mail2.sysu.edu.cn (Z. Huang); huangy2007@jnu.edu.cn (Y. Huang); wangwh9@mail2.sysu.edu.cn (W. Wang); wangwh37@mail2.sysu.edu.cn (W. Wang); dangshsh@mail2.sysu.edu.cn (S. Dang); lich27@sysu.edu.cn (C. Li); mach6@mail2.sysu.edu.cn (C. Ma); zhaozy7@mail2.sysu.edu.cn (Z. Zhao); panxin2@mail.sysu.edu.cn (X. Pan); chuanbin_wu@126.com (C. Wu)

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the δ values between two molecules ($\Delta\delta$) indicated the molecular miscibility [44].

2.4. ACQ property verification of P2 and P4

2.4.1. Spectroscopic transition

The spectroscopic transition of P2 and P4 in a water-fraction-dependent manner [45] was investigated. The UV absorption spectra, fluorescence excitation spectra and fluorescence emission spectra of P2 and P4 in 0–97.5% (*v*/v) water-acetonitrile cosolvent were obtained. The spectra were recorded immediately after sample preparation.

2.4.2. Nanostructure integrity

The nanostructure integrity of P2-SLNS and P4-SLNS was evaluated over a 10-day period. Specifically, probe-containing SLNS systems were stored on the benchtop, and stability, DL and EE were investigated, along with probe leakage into the outer water phase and fluorescence signal retention.

2.4.3. Fluorescence loss in biological matrices

P2-SLNS and P4-SLNS were incubated in a 10-fold volume of phosphate-buffered saline (PBS, Thermo Fisher Scientific Inc., Waltham, MA), plasma, bronchoalveolar lavage fluid (BALF) or lung homogenate. For PBS, the incubation time was 10 days; for the biological matrices, 48-h incubations were performed. At predetermined time intervals, the fluorescence intensity was measured with a NightOWL II LB983 imager.

2.4.4. Quenching triggered by in-vitro lipolysis

A modified pH-controlled in-vitro lipolysis assay [46,47] was employed in this study. In a water bath, the SLNS systems were added to a Tris-maleate buffer solution (Lvye Biotechnology, Jiangsu, China), and the pH was adjusted to 7.50. Pancreatic enzyme solution (Sigma-Aldrich, Inc., St. Louis, MO) preheated to the same temperature was added to activate lipolysis. The pH was maintained at 7.50 with sodium hydroxide (NaOH) solution and was detected by a FiveEasy Plus pH-meter (Mettler-Toledo, LLC, Columbus, OH). The consumption of NaOH solution was recorded. To test the quenching effect triggered by in-vitro lipolysis, the lipolysis inhibitor 4-bromophenylboronic acid (Aladdin Industrial, Inc., Shanghai, China) was added at predetermined time points to suspend the reaction, and the fluorescence intensity of P2-SLNS and P4-SLNS was measured. The fluorescence intensity was plotted versus NaOH consumption, and linear regression was performed [25].

2.4.5. Rekindling effect

Quenched P2 and P4 (P2 or P4 in 97.5% water-acetonitrile cosolvent) were incubated in different organic solvents and aqueous systems. Fluorescence rekindling was demonstrated by a reignited fluorescence signal compared to the fluorescence emitted by the acetonitrile solutions of P2 and P4. Particularly, the rekindling effect of different concentrations of T80 (0.0036–0.92%, *w/v*) was explored, as T80 was the surfactant in SLNS systems.

2.5. In vivo fate of SLNS systems after pulmonary delivery

2.5.1. Animals

Male BALB/c mice weighing 24–28 g were used to study the in-vivo fate. The mice were supplied by Guangdong Medical Laboratory Animal Center (Guangdong, China). All protocols were designed and performed in compliance with related regulations and were approved by the Laboratory Animals Ethics Committee of Sun Yat-sen University (No. SYSU-IACUC-2019-000008 and SYSU-IACUC-2019-000104).

2.5.2. Protocols

An HRH MAG-4 device (Huironghe Technology Co., Ltd., Beijing, China) was used for endotracheal aerosolization in the BALB/c mice. The administration was conducted as reported in a previous work with some modifications [48,49]. The trachea of anesthetized BALB/c mice were surgically exposed, and the delivery tube of HRH MAG-4 was gently intubated. A single 50 μL dose of P2-SLNS systems, DiR-SLNS systems or quenched P2 was insufflated. The surgery wound was sutured and covered by an aseptic gauze.

For the in-vivo bioimaging, the administered BALB/c mice were fixed in the NightOWL II LB983 sample chamber. Fluorescence images were captured at predetermined time intervals. For the ex-vivo bioimaging, the fluorescence signals of the lung and other critical organs (heart, liver, spleen and kidney) were determined. At predetermined time intervals, the BALB/c mice were sacrificed to harvest the lung, and heart, liver, spleen and kidney. Any remaining blood on the organs was removed prior to photographing.

2.5.3. Data processing

The overall fluorescence signal and area-normalized signal were adopted for quantitative comparison. In the in-vivo and ex-vivo bioimaging of the lung, for each time interval, the fluorescence intensity relative to the first time interval (0 h for in-vivo bioimaging and 0.25 h for ex-vivo bioimaging) was plotted versus time and subsequently applied to pharmacokinetics analysis using WinNonlin 5.0.1 software. Pharmacokinetics parameters were calculated including the half-life ($T_{1/2}$), mean retention time ($MRT_{0-\infty}$) and area under the curve (AUC_{0-t}). The correlation between $T_{1/2}$ or $MRT_{0-\infty}$ and particle size of the SLNS systems was established by linear regression. Additionally, the correlation between ex-vivo intensity and in-vivo intensity was assessed by nonlinear regression.

2.6. Statistical information

All experiments were carried out in triplicate or more, if available. Two-tailed *t*-tests or one-way ANOVA followed by Dunnett's test were utilized for statistical analysis. In all cases, $p < .05$ was regarded as statistically significant.

3. Results and discussion

This study aimed to elucidate the relationship between the particle size and the lung retention time of NC after pulmonary delivery, while also confirming the integrity of NC. In-depth knowledge of such a relationship could enable the rational design of NC for pulmonary delivery. SLNS systems with different particle sizes were selected as the model NC. The aza-BODIPY-structured ACQ probes (P2 and P4) were used as a distinguishable bioimaging tool, and non-water-quenching probe DiR served as a reference.

3.1. Physicochemical attributes and nanostructure of SLNS systems

The feasibility of applying the P2, P4 and DiR probes in bioimaging applications was confirmed by investigating their photophysical properties and photostability (Appendix A Section 1–3 and Appendix B Section 3–13). These probes were then encapsulated within the model NC (SLNS systems). Probe-containing SLNS systems were fabricated by the HPH method (Fig. 1A). To investigate the correlation between particle size and lung retention time, SLNS1 ~ SLNS4 were produced, which had increasing particle sizes. Herein, the physiochemical attributes and nanostructure of the SLNS systems were delineated because they exerted a significant impact on the in-vivo fate [50].

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