

# In vivo Pharmacokinetics and in vitro Release of Imatinib Mesylate-Loaded Liposomes for Pulmonary Delivery

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Hongfei Xu<sup>1,\*</sup>

Hongyu Ji<sup>1,2,\*</sup>

Zerong Li<sup>1,3</sup>

Wenmei Qiao<sup>1,4</sup>

Chenghao Wang<sup>1</sup>

Jingling Tang<sup>1</sup>

<sup>1</sup>Department of Pharmaceutics, School of Pharmacy, Harbin Medical University, Harbin, 150086, People's Republic of China; <sup>2</sup>Department of Pharmacy, The Second Affiliated Hospital, Harbin Medical University, Harbin, 150086, People's Republic of China; <sup>3</sup>Department of Pharmacy, The Second People's Hospital of Shenzhen, The First Affiliated Hospital of Shenzhen University, Shenzhen, 518028, People's Republic of China; <sup>4</sup>Department of Pharmacy, Shenzhen Luohu Hospital Group-Shenzhen Luohu Traditional Chinese Medicine Hospital, Shanghai University of Traditional Chinese Medicine-Shenzhen Hospital, Shenzhen, 518001, People's Republic of China

\*These authors contributed equally to this work

**Background:** Pulmonary arterial hypertension (PAH) is characterized by abnormal proliferation of vascular endothelial and smooth muscle cells and causes occlusion of pulmonary arterioles that eventually results in right heart failure and death. The platelet-derived growth factor (PDGF) plays a prominent role in abnormal remodeling of pulmonary resistance vessels. Imatinib mesylate (IM), a PDGF-receptor tyrosine kinase inhibitor, was able to ameliorate PAH by reversing pulmonary vascular remodeling.

**Methods:** In the present study, IM-loaded liposomes (IM-LPs) were developed and administered via the pulmonary route to delay the drug release and improve patient compliance for the treatment of PAH. The IM-LPs were prepared by the transmembrane gradient method with the spherical vesicles. The compatibility of the IM-LPs was studied by determining the viability of pulmonary arterial smooth muscle cells (PASMCS). Particle uptake by rat PASMCS was evaluated by incubating the particles with rat PASMCS. Pharmacokinetic studies were performed in male SD rats.

**Results:** The IM-LPs showed an average size of  $101.6 \pm 50.80$  nm with a zeta potential value of  $19.66 \pm 0.55$  mV, a PDI of 0.250 and  $81.96\% \pm 0.98\%$  drug entrapment efficiency, meanwhile displayed a sustained release profile. Liposomes obviously increased intracellular accumulation of Rhodamine B by PASMCS using the fluorescence microscopic. Following intratracheal administration to rats, IM-LPs not only extended the half-life of IM, but also prolonged retention of IM compared with plain IM solution after intratracheal and intravenous administration.

**Conclusion:** The study show potential applications of the LPs for pulmonary delivery of IM and the method for the development of LPs in sustained release of IM for better therapeutic outcomes. Conclusively, the prepared IM-LPs were well designed in nanosized ranges and may be a promising formulation for pulmonary delivery of IM.

**Keywords:** imatinib mesylate, pulmonary arterial hypertension, liposomes, pulmonary delivery, pharmacokinetics

## Introduction

Pulmonary arterial hypertension (PAH) is characterized by vascular proliferation and remodeling of small pulmonary arteries resulting in increased pulmonary vascular resistance (PVR) and right heart failure.<sup>1</sup> The multiple factors including pulmonary vasoconstriction, endothelial cell dysfunction, pulmonary arterial smooth muscle cells (PASMCS) proliferation, inflammation, and in situ thrombosis play an important role in the mechanism of PAH.<sup>2,3</sup> Currently, the therapeutic drugs for PAH such as endothelin receptor antagonists, phosphodiesterase type 5

Correspondence: Jingling Tang  
Department of Pharmaceutics, School of Pharmacy, Harbin Medical University, Harbin, 150086, People's Republic of China  
Tel +86-0451-86660103  
Fax +86-0451-86664073  
Email tangjl430@hotmail.com

inhibitors, and prostacyclin analogues either alone or in combination, are considered primary treatments.<sup>4-9</sup> However, their full therapeutic abilities are reduced by medication non-compliance and side effects, and PAH is still a fatal disease in many patients.<sup>10,11</sup> Remodeling of the pulmonary artery by an inappropriate increase of PASMCs is the main problem in the treatment of PAH. The reverse remodeling with anti-proliferative and pro-apoptotic agents for PASMCs is required for the effective treatment.

Recent studies have indicated the receptor of tyrosine kinases play a crucial role in the development and progression of pulmonary vascular remodeling. In particular, platelet-derived growth factor (PDGF) plays a prominent role in abnormal remodeling of pulmonary resistance vessels.<sup>12-14</sup> Imatinib mesylate (IM), a PDGF-receptor tyrosine kinase inhibitor, was able to reverse pulmonary vascular remodeling in rats with monocrotaline-induced PAH, as well as in mice with chronic hypoxia-induced PAH.<sup>14,15</sup> IM has anti-proliferative and pro-apoptotic effects on IPAH-PASMCs stimulated with (PDGF)-BB.<sup>14,15</sup> A randomized, double blind, placebo-controlled trial showed that IM improved exercise capacity and hemodynamics in patients with severe PAH, but that serious adverse events and drug discontinuations were common with this treatment.<sup>16</sup> Since systemic administration of IM causes serious adverse events, IM has not been approved for the treatment of PAH until now. The development of a new route of administration for IM is required. Akagi et al examined the efficacy of imatinib-incorporated PLGA NPs (Ima-NPs) in the monocrotaline-induced rat model of PAH and in human PAH-PASMCs.<sup>14,15,17</sup> After a single intratracheal administration, Ima-NPs suppressed the development of monocrotaline-induced PAH in rats. The release of imatinib from NPs in PAH-PASMCs was sustained, and proliferation of the cells was inhibited by Ima-NPs.<sup>14,15</sup>

Pulmonary delivery is suited for PAH treatment because of the large surface area of the lungs for improving the rapid uptake of drugs, the short distance between the site of absorption and the pulmonary artery,<sup>18-21</sup> and the reduction in drug exposure to the systemic circulation for minimizing the dose-related side effects.<sup>22</sup> Following pulmonary administration, liposomes can prolong the residence of the entrapped drug and minimize the absorption to the systemic circulation, resulting in prolonged local therapeutic effect of the drug and reduced potential of systemic adverse effects.<sup>23-29</sup>

The purpose of this study is to design and develop imatinib mesylate-loaded liposomes (IM-LPs) and administered via the intratracheal administration for the treatment of PAH. IM-LPs were prepared and characterized by their physicochemical properties. Rhodamine B (RhoB), a fluorescent probe, was incorporated into liposomes to evaluate the cellular uptake in PASMCs using fluorescence microscope. The pharmacokinetics of IM for IM-LPs by intratracheal or intravenous administration was studied in healthy rats.

## Materials and Methods

### Materials

Cholesterol (95%) was purchased from Alfa Aesar Chemicals Co., Ltd (Shanghai, China). Soy lecithin (SL, CS-95) was from A.V.T. Pharmaceutical Co., Ltd (Shanghai, China). Imatinib mesylate was supplied by Shanghai Yingrui Pharmaceutical Technology Co., Ltd (Shanghai, China). Rhodamine B (RhoB), DAPI (4',6-diamidino-2-Phenylindole, Dihydrochloride) and Sephadex G-50 medium were purchased from Solarbio Technology Co., Ltd (Beijing, China). All other chemicals including methanol, acetonitrile, phosphate buffered saline (PBS 1×), ammonium sulfate, dimethyl sulfoxide (DMSO), potassium phosphate monobasic (KH<sub>2</sub>PO<sub>4</sub>) were of analytical grade and all chemicals were used without further purification.

The PASMCs of rats were provided by the Department of Biopharmaceutical of School of Pharmacy of Harbin Medical University. Male Sprague-Dawley (SD) rats (200–250g) were bought from the Experimental Animal Center of the Second Affiliated Hospital of Harbin Medical University (Heilongjiang, China). All animal procedures were approved by the Animal Ethics Committee of School of Pharmacy of Harbin Medical University, China. The guidelines GB/T 35,892–2018 was followed for the welfare of the laboratory animals.

### Optimization and Preparation of IM-LPs

IM-LPs were prepared using SL and cholesterol at a mass ratio of 4:1, and the lipid concentration was 35 mg/mL. Briefly, the lipids (SL and cholesterol) were dissolved in ethanol, which was removed by the rotary evaporation at 40°C. The dried lipid film, formed in a round-bottomed flask, was rehydrated with 300 mM ammonium sulfate (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> solution for 30 minutes at 50°C. Then, the rehydrated film was sonicated for minimizing particle

size. The transmembrane gradient was formed by exchanging external ammonium sulfate with glucose solution using dynamic dialysis. IM was added to the above prepared liposomes followed by 20 minutes of incubation at 50°C for IM-LPs and IM was incorporated by active loading. RhoB-loaded liposomes (RhoB-LPs) were prepared in the same way. IM-LPs and RhoB-LPs were stored at 4 °C for further studies.

## HPLC Analysis of IM

The drug content was analyzed by reversed phase HPLC using Agilent 1260 series system that was equipped with a quaternary pump, a diode array detector, an autosampler and workstation. The sample (20 µL) was injected into a Diamonsil™ C<sub>18</sub> column (250 mm × 4.6 mm, 5 µm). The mobile phase was composed of 0.05 mM KH<sub>2</sub>PO<sub>4</sub> solutions which was adjusted to pH 3.0 using ortho-phosphoric acid 85% and acetonitrile (75: 25, v/v) and was run at a flow rate of 1 mL/min. UV detection was set at 265 nm and analysis was carried out at 30°C.

## Physicochemical Characterization

### Characterization of IM-LPs

The particle size, polydispersity index (PDI), and zeta potential of the freshly prepared IM-LPs were measured using a Zetasizer (Nano ZS90, Malvern Instruments Inc, Worcestershire, UK) at 25°C. The samples were diluted with double-distilled water, and all measurements were performed in triplicate.

Particle size and morphology were determined using a transmission electron microscope (TEM). A drop of the diluted IM-LPs was applied onto a copper grid covered with nitrocellulose, excess liquid was then wicked off. Then the sample was negatively stained with 2% (w) phosphotungstic acid, followed by overnight air-drying at room temperature. Images were acquired using a TEM (Tecnai G2F30; FEI Co., Hillsboro, OR).

### Determination of Encapsulation Efficiency (EE) of IM-LPs

The encapsulation efficiency (EE) of IM-LPs was determined by the method of gel chromatography that separating the unencapsulated IM using a Sephadex gel-50 chromatography (1.5 × 25.0 cm). In brief, 0.2 mL of IM-LPs was loaded onto a Sephadex gel-50 chromatography with PBS (pH 7.4) as the eluant. The light blue opalescent eluent was collected from the column, and another 0.2 mL sample was directly diluted with PBS to equal volume.

Then, the sample (1 mL) was diluted to 5.0 mL with methanol. IM was determined by HPLC method as described in the previous section. The EE of IM-LPs was calculated as follow:  $EE (\%) = W_L / W_T \times 100\%$ , where  $W_L$  is the determined quantity of IM loaded in the liposomes subsequent to passing over the gel column, and  $W_T$  is the determined quantity of IM in the liposomes prior to passing over the gel column.

### In vitro Release of IM from IM-LPs

In vitro IM release from freshly prepared IM-LPs was investigated by the dialysis bag technique with PBS (pH 5.5) as the receiving medium within 48 hours. In brief, IM-LPs or IM solutions (1 mL) were transferred to the pre-treated dialysis bags (molecular weight cut off 8–14 kDa), which were placed in the receiving medium (80 mL) at  $37 \pm 1^\circ\text{C}$  under stirring at 100 r/min. Aliquots of the receiving medium (1 mL) were drawn at predetermined time intervals (0.25, 0.5, 1, 2, 4, 6, 8, 10, 24 and 48 h) and passed through a 0.45 µm filter membrane, while the fresh medium (1 mL) was added to the receiving medium. The concentration of the IM within the collected supernatant was determined by HPLC method as described in the previous section “HPLC analysis of IM”.

## Cell Viability Study

The cytotoxicity of the optimized IM-LPs was investigated using a MTT assay in the PSMCs. The PSMCs was cultured in Dulbecco's Modified Eagle Medium (DMEM) (HyClone, Logan, UT) supplemented of 20% fetal bovine serum (Everygreen, Zhejiang, China), 100 U/mL of penicillin G sodium, and 100 µg/mL of streptomycin sulfate (Beyotime, Shanghai, China). Cells were maintained in a humidified incubator at 37°C with 5% CO<sub>2</sub>. Briefly, cells were seeded in a 96 well plates at a density of  $5 \times 10^4$  cells per well and incubated in DMEM for 24 h attachment. Then, the cells were treated with (i) saline (negative control), (ii) 0.1% sodium dodecyl sulfate (0.1%SDS, positive control), (iii) plain IM (25 µM), (iv) IM-LPs (equivalent to 25 µM IM) and (v) plain liposomes. Following a 24 h period of incubation, the cells were washed with PBS and then incubated with 20 µL MTT solution (5 mg/mL in sterile PBS) for 4 h at 37°C. The formazan crystals were solubilized in 100 µL DMSO with appropriate shaking on a plate shaker for 10 min. The absorbance value was read on a microplate reader at 570 nm (Tecan Infinite 200 PRO, Austria, Switzerland). The cell viability was calculated as follow:  $\text{Cell viability } (\%) = (A_{\text{sample}} - A_{\text{blank}}) / (A_{\text{control}} -$

$A_{\text{blank}} \times 100\%$ ;  $A_{\text{sample}}$  is the absorbance for the treated cells,  $A_{\text{control}}$  is the absorbance for saline treatment and  $A_{\text{blank}}$  is the absorbance for no treatment.

## Cellular Uptake of Liposomes

For the cellular uptake study, PSMCs were used to study the cellular uptake of liposomes. RhoB-LPs was used instead of IM-LPs as a fluorescent probe to observe the cellular uptake. Briefly, the cells were seeded in a 12 well plates at a density of  $1 \times 10^4$  cells per well and incubated in DMEM medium for 24 h attachment. Then, the cells were incubated with RhoB-LPs for 2 h. After incubation, the cells were washed with PBS for three times and stained with 4', 6-diamidino-2-Phenylindole, Dihydrochloride (DAPI). The cells were then viewed under a fluorescent microscope (Olympus, Japan).

## Pharmacokinetics Study

### Dosage Regimen and Plasma Samples

The rats were randomly divided into three groups and anesthetized by hydrated chloral enema ( $n=6$ ). IM solution (10 mg/kg) was given to the rats of the first group via the penile vein (i.v. plain IM). For the other two group, after the rats were anesthetized, a syringe fitted with a 22-gauge intravenous catheter was inserted almost to the bottom of the trachea.<sup>30</sup> IM solutions or IM-LPs were given to at the dosage of 10 mg/kg by intratracheal instillation (I.T.). Blood samples ( $\sim 500 \mu\text{L}$ ) were collected into heparinized tubes at 0.083, 0.25, 0.5, 1, 2, 4, 6, 8 and 10 h for i.v. plain IM group, at 0, 0.083, 0.25, 0.5, 1, 2, 4, 6, 8, 10 and 24 h for I.T. plain IM group and I.T. IM-LPs group. The plasma was separated by centrifugation at 4000 r/min for 10 min and stored at  $-20^\circ\text{C}$  until further analysis.

### Sample Treatment and Statistical Analysis

An aliquot of 20  $\mu\text{L}$  of the internal standard (vardenafil) and 20  $\mu\text{L}$  methanol was added to a 100  $\mu\text{L}$  plasma sample. The mixture was treated with 2 mL extraction solvent (Dichloromethane: tert-butyl methyl ether = 1: 3, v/v) then vortexed for 5 min. After centrifugation for 10 min at 4000 r/min, the organic upper layers were transferred to a new tube and dried under nitrogen stream at  $40^\circ\text{C}$  using N-EVAP (L-119A, Laiheng, Beijing, China). The residue was reconstituted in 100  $\mu\text{L}$  methanol by sonification for 3 min, vortex mixing, centrifugation at 3000 r/min for 10 min and the vials were placed in the HPLC autosampler. Subsequently, 20  $\mu\text{L}$  aliquots of each supernatant were analyzed by HPLC as described in the

previous HPLC Analysis of IM. The curve of the IM content in plasma was determined by the internal standard method with a good linearity from 0.1  $\mu\text{g/mL}$  to 8  $\mu\text{g/mL}$  with a correlation coefficient of 0.9994. The absolute recoveries of IM and internal standard from blank plasma were  $86.12 \pm 4.29\%$  and  $85.16 \pm 0.93\%$ , respectively.

The pharmacokinetic parameters were fitting by DAS (The drug and statistics software, version 2.0). The absolute bioavailability (F) of IM was calculated as follows:

$$F = \frac{AUC_{I.T.} \bullet D_{i.v.}}{AUC_{i.v.} \bullet D_{I.T.}} \times 100\%$$

Where  $AUC_{I.T.}$  and  $AUC_{i.v.}$  refers to the AUC after administration by I.T. and i.v., and  $D_{I.T.}$  and  $D_{i.v.}$  is doses of administration by I.T. and i.v., respectively.

All data were examined using GraphPad Prism 5.0 software and expressed as mean  $\pm$  standard deviation. Student's *t*-test was statistically significant at *P*-values  $< 0.05$ .

## Results and Discussion

### Optimization and Physicochemical

#### Characterization of IM-LPs

##### Drug Entrapment Efficiency and Loading Efficiency

In the present study a natural phospholipid, the SL, was chosen as the bilayer components of liposome for getting a better rigidity/fluidity and providing much more permeable liposomes and facilitating large-scale industrial production compared with other saturated phospholipids.<sup>31</sup> In the present study, the active loading using  $(\text{NH}_4)_2\text{SO}_4$  based transmembrane gradient method was developed for improving the entrapment efficiency of IM. Ye et al had reported the preparation of folate-targeted liposomes containing IM by ammonium sulfate gradient method with the entrapment efficiency of up to 90%.<sup>32</sup>

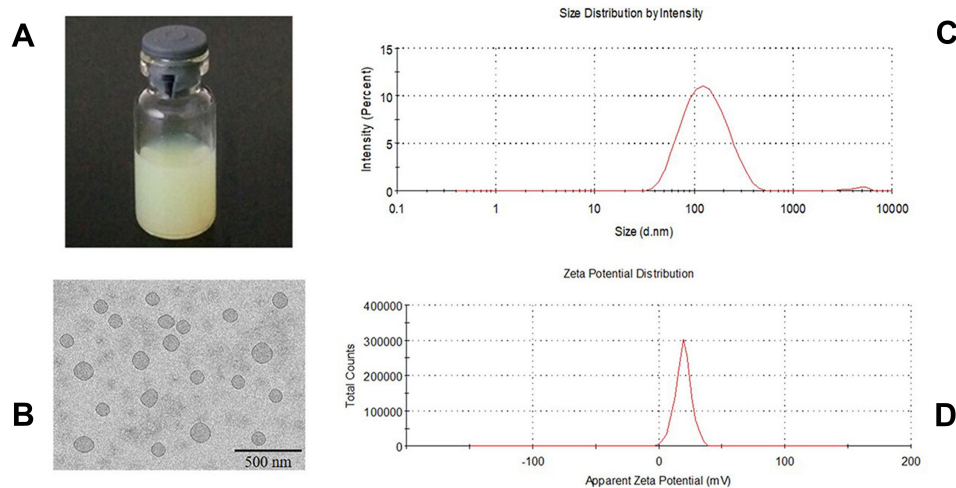
In this method, the presence of ammonium sulfate in the core of liposomes produces an excess availability of protons inside the vesicle because of removal of ammonium sulfate from the extraliposomal compartment resulting in a proton gradient across the liposomal membrane.<sup>33,34</sup> Unprotonated weak bases outside the vesicles can easily permeate through liposomal membranes. Once inside the vesicles, they are protonated in  $\text{H}^+$  rich environment and thereby trapped in the aqueous core of the vesicles.<sup>33,34</sup> Imatinib, which has four protonizable amine functional groups, can cross the lipid bilayer and be entrapped in the liposomal core due to complexation with sulfate ion.<sup>32</sup> When the gradient is constant, the

active drug loading is a temperature-dependent drug loading method. When the drug enters the internal water phase through the bilayer membrane in a molecular state, it must pass an energy barrier (activation energy  $E_a$ ). Only when the temperature reaches a certain temperature, the drug can overcome this activation energy and enter the internal water phase. Excessive temperature will lead to oxidation of phospholipids, affecting the stability of liposomes. The entrapment efficiency was reduced after incubation for

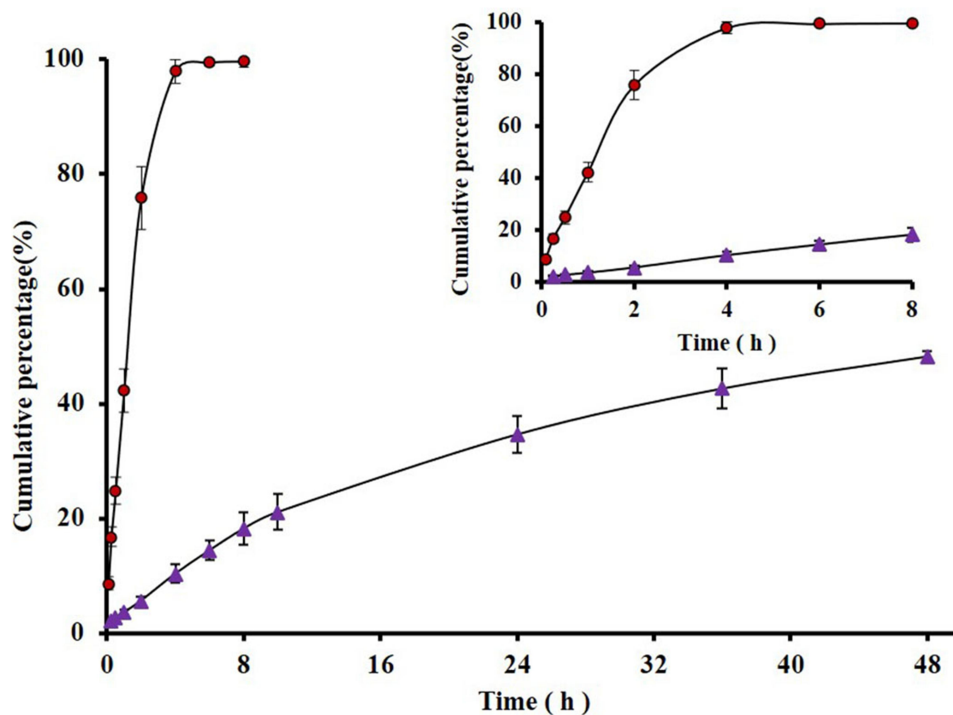
more than 20 minutes. Therefore, the optimized IM-LPs were incubated for 20 min at 50 °C with light yellow-green appearance (Figure 1A),  $EE$  of  $81.96 \pm 0.98\%$  and  $LE$  of  $4.80 \pm 0.05$  mg/mL.

### Particle Size and Morphology

TEM image exhibited a regular spherical shape with diameters about 100 nm (Figure 1B). The average particle size of optimized IM-LPs was  $101.6 \pm 50.80$  nm with a



**Figure 1** Characterization of IM-LPs. (A) Transmission electron microscopic image, (B) The appearance of IM-LPs, (C) Size distribution, (D) Zeta potential.



**Figure 2** In vitro release profiles of plain IM and IM-LPs in pH 5.5 PBS at 37°C (Data represent mean  $\pm$  SD,  $n = 6$ ). (●) plain IM; (▲) IM-LPs.

PDI of 0.250 (Figure 1C). The liposomes with the smaller size are less likely to be phagocytosed by macrophages.<sup>35</sup> Surface charge can keep particles separated and prevent particle aggregation, which is an important factor for colloidal stability.<sup>35,36</sup> In this study, the zeta potential of the optimized liposomes was about  $19.66 \pm 0.55\text{mV}$  (Figure 1D), which is adequate for the stability of the formulation.

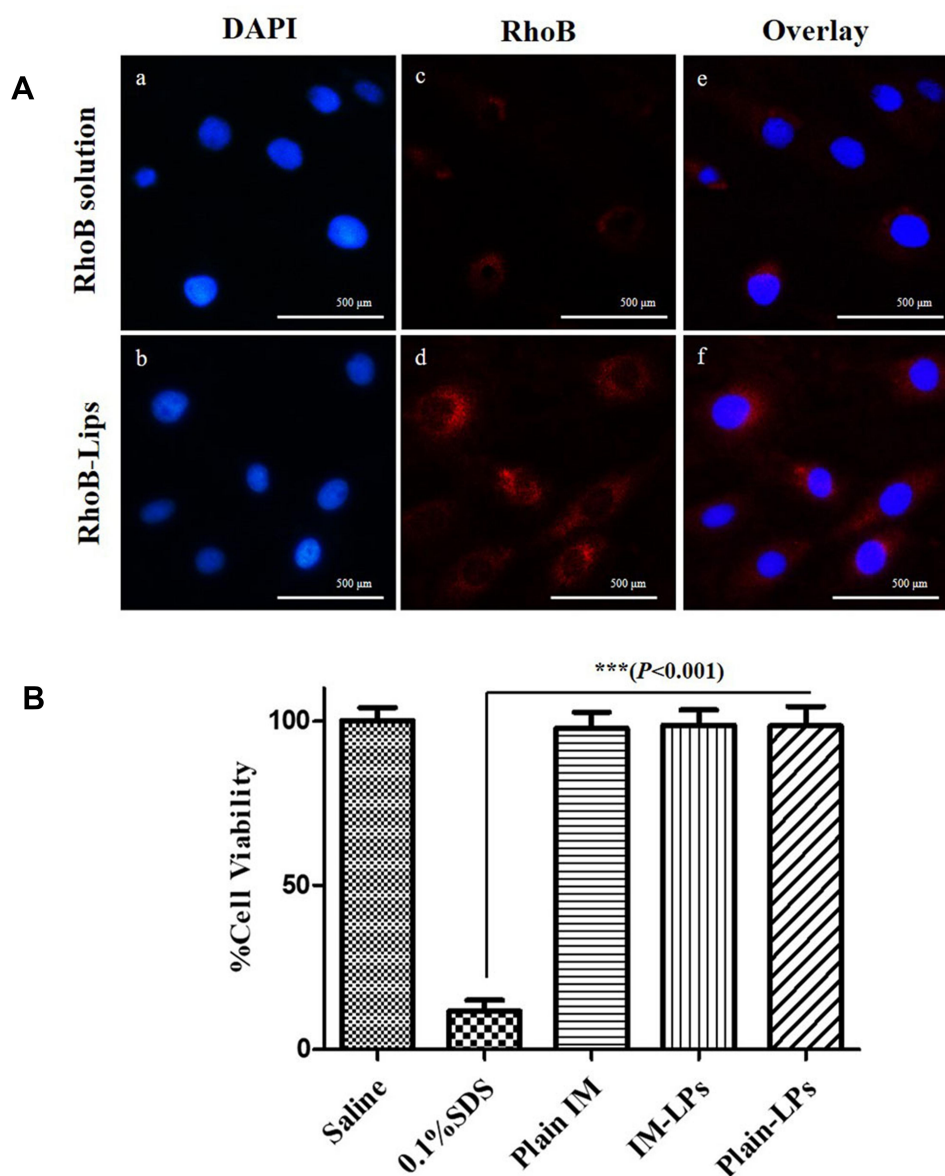
### In vitro Drug Release

The in vitro release profiles of IM-LPs were evaluated in pH 5.5 PBS buffer at 37°C. When the release of plain IM

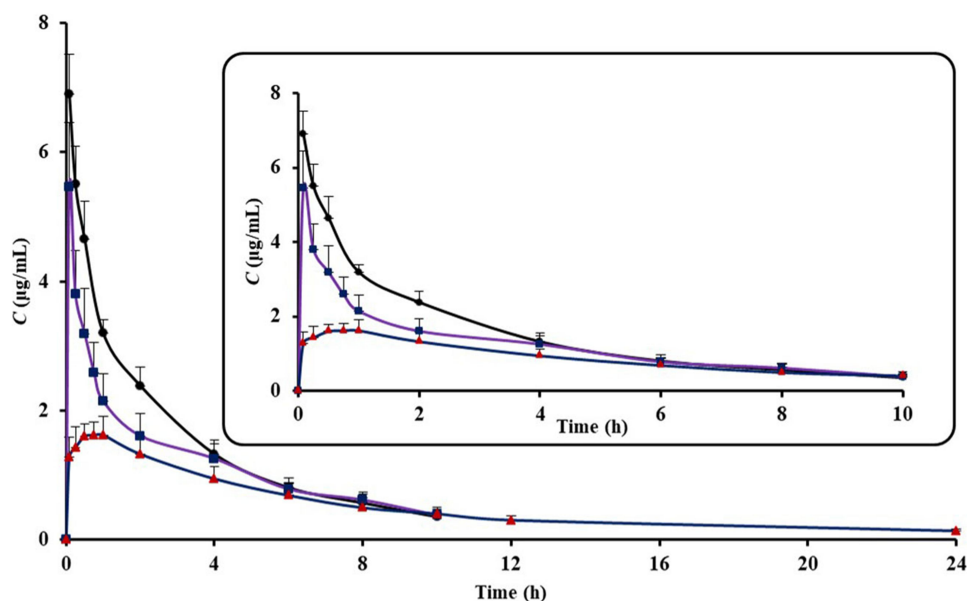
was evaluated using dialysis bags as barriers, >90% drug was available in the receiver chamber only after 8 h. However, only  $48.3 \pm 0.97\%$  drug was released from IM-LPs after 48 h (Figure 2). It was indicated that the drug was slowly released from the liposomes, probably due to drug diffusion from the lipid bilayer instead of being released due to disruption of liposomes.<sup>35</sup>

### Uptake of Liposomes by PSMCs

The site of action of IM is the pulmonary arterial smooth muscle cells and the target receptor, PDGFR, is expressed



**Figure 3 (A)** Representative fluorescence microscopic images showing the uptake of liposomes by the PSMCs. (Up) RhoB solution; (Down) RhoB-LPs. Fluorescent images of the cell nucleus stained with DAPI (a and b), fluorescent images of the RhoB (c and d), overlay (e and f), **(B)** The viability of PSMCs upon incubation with IM-LPs or plain drug (Data represent mean  $\pm$  SD, n = 6). \*\*\*Results are significantly different ( $P < 0.001$ ).



**Figure 4** The plasma concentration-time profiles of IM (Data represent mean  $\pm$  SD,  $n = 6$ ). (▲) I.T. IM- LPs, (■) I.T. IM solution, (●) I.V. IM solution.

on this cell surface. RhoB (a fluorescent probe) was incorporated into liposomes to evaluate the cellular uptake in PASCs by the fluorescence microscope (Figure 3A). PASCs were incubated for 2 h with RhoB-loaded liposomes (red), a significantly higher uptake was observed compared with plain RhoB solution, indicating that liposomal formulations can promote the uptake of drugs by PASCs.

### Cell Viability Study

The effect of IM and IM-LPs on the viability of PASCs was determined by an MTT assay. The PASCs were treated with saline (negative control) and 0.1% *w/v* SDS (positive control) with cell viability of  $98.25 \pm 3.85\%$  and  $11.51 \pm 3.18\%$ , respectively (Figure 3B). The cell viability of PASCs was all  $>95\%$  when treated with plain IM, IM-LPs (equivalent to  $25 \mu\text{M}$  IM) and blank liposomes for 24 h. Satoshi et al have reported that after treatment with PDGF-BB promoted proliferating PASCs then treat with IM at a concentration of  $1\text{--}10 \mu\text{g/mL}$  for 24 h, a significant

inhibitory effect was produced.<sup>37</sup> The IM concentration of the cytotoxicity experiment in this study was  $14.74 \mu\text{g/mL}$  ( $25 \mu\text{M}$ ), indicating that IM-LPs was not toxicity to PASCs, and may have a proliferation inhibitory effect on abnormally proliferating PASCs.<sup>25</sup>

### Pharmacokinetics Study

The plasma concentration-time profiles of IM following i. v. of IM solution, I.T. of IM solution and IM-LPs were shown in Figure 4. The main pharmacokinetic parameters derived from plasma concentration-time profiles were summarized in Table 1. The  $MRT_{0 \rightarrow \infty}$  of IM after I.T. of IM-LPs, IM solution and tail vein injection of IM solution were  $10.38 \pm 1.62 \text{ h}$ ,  $4.97 \pm 0.72 \text{ h}$  and  $4.05 \pm 0.58 \text{ h}$ , respectively. It shows that the retention time of IM after I. T. is significant prolonged ( $P < 0.05$ ). The significant difference was also observed for the  $t_{1/2\beta}$ .  $t_{1/2\beta}$  of IM for plain IM after I.T. was approximately 1.29-fold that of plain IM after i.v. ( $t_{1/2\beta} = 4.08 \pm 1.01 \text{ h}$  for IT,  $t_{1/2\beta} = 3.15 \pm 0.47 \text{ h}$  for i.v.). However, a significant increase in the

**Table 1** The Plasma Pharmacokinetic Parameters of IM Following Intravenous and Intratracheal Administration of Plain IM and IM-LPs to Rats

Treatment	$C_{\text{max}}$ ( $\mu\text{g/mL}$ )	$t_{1/2\beta}$ (h)	$AUC_{0 \rightarrow \infty}$ ( $\mu\text{g/mL h}$ )	$MRT_{0 \rightarrow \infty}$ (h)	Bioavailability (%)
Plain IM I.V.	—	$3.15 \pm 0.47$	$17.42 \pm 1.94$	$3.82 \pm 0.45$	—
Plain IM I.T.	—	$4.08 \pm 1.01$	$14.54 \pm 1.55$	$4.97 \pm 0.72$	83.46%
IM-LPs I.T.	$1.79 \pm 0.19$	$12.68 \pm 4.43^*$	$13.51 \pm 1.54$	$10.38 \pm 1.62^*$	77.55%

**Notes:** \*Results are significantly different ( $P < 0.05$ ), IM-LPs were compared with plain IM. Data represent mean  $\pm$  SD,  $n = 6$ .

half-life for I.T. of IM-LPs was observed, about 3–4 fold higher than plain IM after i.v. and I.T. ( $12.68 \pm 4.43$  h,  $P < 0.05$ , in Table 1). Differences absorption profiles between plain IM and IM-LPs can be attributed to liposomes can prolong the residence of the entrapped drug in the “deep lung” and minimize the absorption to the systemic circulation.

## Conclusion

In the present study, IM-loaded liposomes (IM-LPs) were successfully developed with a narrow size distribution, high entrapment efficacy and a sustained release profile. The uptake of RhoB was enhanced by the RhoB loaded liposomes in PSMCs by the fluorescence microscope. Following intratracheal administration to rats, IM-LPs not only extended the half-life of IM, but also prolonged retention of IM. In summary, liposomes may be promising carriers for pulmonary delivery of IM to delay the drug release and improve patient compliance for the treatment of PAH.

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## Disclosure

The authors declare no competing financial interest and report no conflicts of interest in this work.

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