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Preparation and Quality Evaluation of Salvianolic Acids and Tanshinones Dry Powder Inhalation

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ABSTRACT

Salvianolic acids and tanshinones both exhibit efficacy in treating idiopathic pulmonary fibrosis (IPF), but their formulation limits their clinical use. This study aimed to prepare the salvianolic acids and tanshinones dry powder for inhalation (SPI) to achieve pulmonary delivery for the treatment of IPF. The variable quantities of salvianolic acids and tanshinones composite powder were optimized using the central composite design-response surface method. Different carriers with various drug-carrier ratios were optimized to prepare SPI. The final optimized formulation of SPI was as follows: InhaLac 230® was selected as the carrier with drug:carrier = 1:6, and the milled lactose InhaLac 400® was added at 5%. The developed SPI characterized with an angle of repose 52.46 ± 1.04°, Carr's index of 34.00 ± 0.50% and showed high lung deposition *in vitro*, indicating the potential of pulmonary delivery for the treatment of IPF.

Introduction

Idiopathic pulmonary fibrosis (IPF) is a diffuse pulmonary inflammatory disease caused by a variety of causes, with the pathologic manifestations of excessive interstitial collagen deposition and excessive proliferation of fibroblasts, finally leading to respiratory failure.[1,2] Although the etiology remains unknown, some scholars believe that the evolution of IPF can be divided into oxidative stress, alveolitis, and fibrosis formation stages.[3] At present, only nintedanib and pirfenidone are approved by Food and Drug Administration to treat IPF. However, they only slow down the decline in lung function. They can neither terminate the progression of the disease nor reverse the condition.[4,5]

Salvia miltiorrhiza is one of the commonly used traditional Chinese medicine for treatment of various fibrosis.[6,7] Salvianolic acids and tanshinones, the main extracts of *S miltiorrhiza*, have a significant antioxidant effect and strong anti-inflammatory activity, respectively.[8-10] Salvianolic acid B is the main monomer component in salvianolic acids, and its content can be 80%. It can alleviate experimental pulmonary fibrosis both *in vivo* and *in vitro* by inhibiting the transforming growth factor β1 signaling pathway.[11] Tanshinone IIA is the main monomer component in tanshinones, with a content of up to 60%. It can mitigate bleomycin-induced pulmonary fibrosis and suppresses transforming growth factor β1−dependent epithelial to mesenchymal transition of lung alveolar epithelial cells.[12] Both of them have been proven to effectively ameliorate IPF in rats[13] and *in vitro*.[11] *S miltiorrhiza* exhibits potential benefits against IPF, the mechanisms of which appear to involve the regulation of inflammation, oxidant stress, profibrotic signaling pathways, and so forth.

S miltiorrhiza is usually administered orally or intravenously for the treatment of various fibrosis in clinics currently.[14-16] However, both the salvianolic acids and tanshinones which are lipophilic have obvious liver first pass effect and enterohepatic circulation phenomenon, leading to the oral bioavailability less than 5%.[17,18] Although injection can ensure that drugs get into the blood directly to achieve good therapeutic effect, in terms of long-term use for chronic lung disease, the patients show poor compliance due to frequent injection. Pulmonary inhalation shows unique advantages in the treatment of lung disease that can not only ensure drugs releasing in the lung but also keep drugs away from the intestinal and liver metabolism. Dry powder for inhalation (DPI) may be inhaled to the lungs by the patient actively. Compared with aqueous or nonaqueous droplets from nebulizers or metered dose

Abbreviations used: IPF, idiopathic pulmonary fibrosis; SPI, salvianolic acids and tanshinones dry powder for inhalation; STW, salvianolic acids and tanshinones composite powder; DPI, dry powder for inhalation; NGI, Next Generation Impactor; TD, total dose; FPD, fine particle dose.

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Table 1
In Vitro Deposition Properties of Salvianolic Acid B and Tanshinone IIA in Different Formulations With NGI at 60 L/min

Sample	Total Dose (mg)	Delivery Dose (mg)	Delivery Rate (%)	Dosage of Fine Particles (mg)	Fine Particle Fraction (%)
Salvianolic acid B					
STW	9.52	5.61	58.92	1.77	31.59
STW:InhaLac 230®					
1:3	1.67	1.28	76.73	0.42	33.02
1:6	1.06	0.76	71.91	0.27	35.13
1:9	0.65	0.40	61.01	0.10	25.73
STW:InhaLac120®					
1:3	1.80	1.17	65.43	0.35	29.64
1:6	1.01	0.61	60.01	0.18	30.22
1:9	0.66	0.40	61.06	0.11	26.33
Tanshinone IIA					
STW	5.09	2.94	57.76	1.01	34.16
STW:InhaLac230®					
1:3	0.65	0.54	82.30	0.21	38.47
1:6	0.42	0.33	79.96	0.13	38.21
1:9	0.26	0.20	78.74	0.063	30.87
STW:InhaLac120®					
1:3	0.79	0.55	69.59	0.19	35.30
1:6	0.42	0.28	66.97	0.10	36.49
1:9	0.27	0.21	79.49	0.07	31.19

Ten capsules were used for each deposition experiment.

inhalers and other pulmonary administration forms, DPI are more stable, easier to carry and acceptable for patients and do not need propellant.[19] However, the DPI of salvianolic acids and tanshinones has not been studied before.

In this study, we prepared an inhalable powder mixture of salvianolic acids and tanshinones to achieve pulmonary delivery which was expected to treat IPF. The *in vitro* physicochemical characteristic of the salvianolic acids and tanshinones dry powder for inhalation (SPI) was evaluated, such as morphology, flowability, moisture absorption, and *in vitro* deposition.

Materials and Methods

Materials

Total salvianolic acids (containing 80% salvianolic acid B; batch number: 150314; Nanjing Langze Pharmaceutical Company, Nanjing, China), total tanshinone (containing 60% tanshinone IIA; batch number: 141011; Nanjing Langze Pharmaceutical Company), salvianolic acid B (used as reference substance, purity: ≥98%, batch number: 3857/22081; Shanghai Nature-Standard Biotechnologies, Inc., Shanghai, China), Tanshinone IIA (≥98%, batch number: 961/22087; Shanghai Nature-Standard Biotechnologies, Inc.), InhaLac 120®, InhaLac230®, InhaLac 400® (Meggle Pharma, Wasserburg, Germany) and other materials or solvents were obtained from the indicated suppliers.

LC-20A High Performance Liquid Chromatography System (SPD-20A detector; Shimadzu Company, Kyoto, Japan), ultra-low temperature refrigerator (Sanyo Company, Tokyo, Japan), freeze-drying machine (LGJ-12; Beijing Songyuan Huaxing Technology Development Co., Ltd., Beijing China), frequency planetary ball mill (XQM-0.4L; Nanjing Daran Technology Co., Ltd., Nanjing, China), Next-Generation Pharmaceutical Impactor (Copley Scientific Limited Company, Nottingham, UK), laser diffraction size analyzer (BT-2001; Dandong Bettersize Instrument Co., Ltd., Liaoning, China), vacuum drying oven (DZF-6050; Shanghai Boxunshiye Limited Company), and scanning electron microscope (InspectTM S50; FEI).

Methods

Preparation of Composite Micro-Powder

The salvianolic acids and tanshinones composite powder (STW) was obtained according to our previous research.[20] Total salvianolic acids of 4.565 g and 2.435 g of total tanshinones were placed in a 100 mL stainless steel ball mill. The grinding medium was stainless steel ball (Φ5:Φ3 = 1:8), the ratio of ball to material was 17.5, the dispersant was cyclohexane, the slurry concentration was 50%, grinding speed was 250 min/r, grinding time was 3.5 h. Then, the mixture was transferred to a flat weighing bottle and dried under

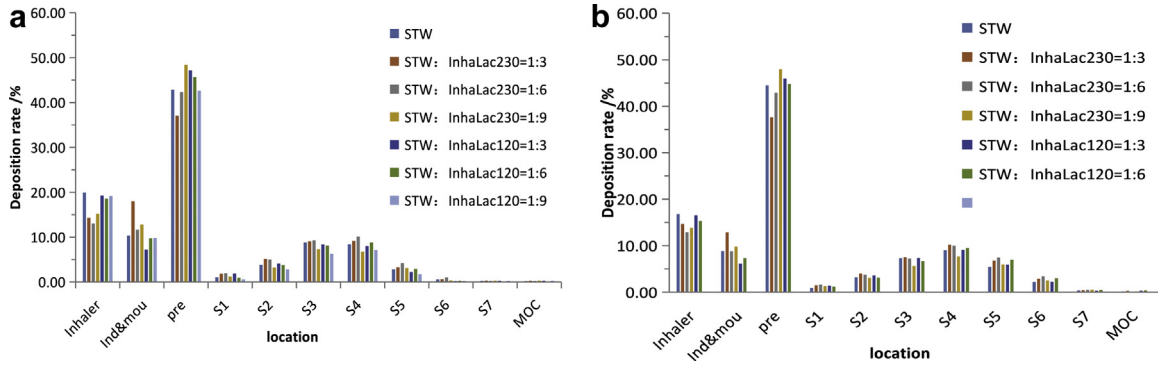

Figure 1. Deposition of salvianolic acid B and tanshinone IIA in different prescriptions with NGI at 60 L/min; (a) salvianolic acid B (b) tanshinone IIA. MOC, micro-orifice collector.