European Journal of Pharmaceutics and Biopharmaceutics 70 (2008) 845–852

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Pulmonary delivery of scutellarin solution and mucoadhesive particles in rats

Xiao-Bin Liu [a], Jun-Xiao Ye [a], Li-Hui Quan [a], Chun-Yu Liu [a], Xu-Liang Deng [b], Ming Yang [c], Yong-Hong Liao [a,*]

[a] Institute of Medicinal Plant Development (IMPLAD), Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, PR China
[b] Center for Biomedical Materials and Tissue Engineering, Peking University, Beijing, PR China
[c] Jiangxi College of Traditional Chinese Medicine, Jiangxi, PR China

ARTICLE INFO

Article history:
Received 19 May 2008
Accepted in revised form 3 July 2008
Available online 17 July 2008

Keywords:
Scutellarin
Flavonoids
Intratracheal spray
Pulmonary delivery
Intravenous delivery
Peroral delivery
Mucociliary transport
Hyaluronan

ABSTRACT

The objectives of this study were to investigate the effects of mucoadhesive excipients on systemic bioavailability of an inhaled drug and to evaluate the feasibility of using the pulmonary route for non-invasive systemic delivery of scutellarin, a poorly orally absorbed flavonoid glucuronide. Following intratracheal spray of the scutellarin solution, the bioavailability was found to be approximately 77% in rats, which was >30-fold higher than that via the peroral route. In addition, the pulmonary absorption of scutellarin appeared to avoid the intestinal first-pass metabolism accompanied by peroral administration. Spray-dried scutellarin particles with the presence of mucoadhesive excipients were found to affect the corresponding mucociliary transport rate (MTR) as evaluated by a frog palate model. The pharmacokinetic results indicated that the magnitude of AUC_{0-480} of intrapulmonary delivered drug particles was not correlated to the fine particle fraction (FPF) but inversely related to the MTR. Incorporating mucoadhesive polymeric mixtures into the scutellarin particles, the MTR decreased by sixfold, and the absolute bioavailability of the drug was found to increase from 70.1% to 97.9% despite a decrease in the FPF. Moreover, *in vitro* results evaluated using Calu-3 and A549 cell lines showed that scutellarin and spray-dried particles with or without the presence of mucoadhesives exhibited no local cell cytotoxic effects in the tested concentration range. In conclusion, the conducting airway is well permeable to scutellarin, and scutellarin may be effectively delivered systemically through inhalation of respirable droplets or particles.

1. Introduction

Scutellarin, 4′,5,6-tetrahydroxyflavone-7-*O*-glucuronide, is the major effective constituent of breviscapine isolated from a Chinese herb, *Erigeron breviscapus* (Vant). Breviscapine has been extensively used in clinic for the acute and long-term treatment of cardiovascular diseases and cerebrovascular injury in China and in most of the commercial formulations, breviscapine contains over 80% of scutellarin [1–3]. Numerous studies have demonstrated that breviscapine possesses a variety of pharmacological effects, such as dilating blood vessel, improving microcirculation, increasing cerebral blood flow, and inhibiting platelet aggregation [4–6]. However, pharmacokinetic studies showed that the oral bioavailability of breviscapine using scutellarin as an indicator was poor, ranging from 0.5% to 5% [7]. As a result, injectables are the main choice of dosage forms for commercial preparations of breviscapine. Nonetheless, therapeutic effects elicited by breviscapine require repeated injection daily for a long time. This is highly inconvenient and results in low patient compliance. Therefore, non-invasive alternative delivery routes are desirable.

Inhalation administration of pharmaceuticals to the pulmonary epithelium for systemic delivery represents a significant opportunity for many classes of drugs, in particular for those with poor oral bioavailability [8]. Compared to peroral delivery, this route of administration eliminates the potential for poor absorption and/or high metabolism in the gastrointestinal tract and first-pass losses in the liver, whereas relative to injection therapy, inhalation therapy is not associated with pain and as a consequence this should improve patient comfort and compliance, leading to improved treatment outcome [10]. The unique features of the lung including large surface area, good vascularization, and ultra-thinness of the alveolar epithelium can facilitate systemic delivery of various pharmaceuticals via inhalation route [9]. As such, most of the previous studies on systemic inhalation delivery pay attention to lung deposition efficiencies, particularly the lower respiratory region (e.g., [8,11]), whereas relatively few studies focus on pharmacokinetic fate of drug molecules deposited in the conducting airways (trachea, bronchi, and bronchioles) [12]. Although it is ideal to achieve a greater percentage of deposition within lung periphery (i.e. alveolar region) for systemic pulmonary delivery, a

* Corresponding author. Institute of Medicinal Plant Development (IMPLAD), Chinese Academy of Medical Sciences and Peking Union Medical College, 151 Malianwa North Road, Haidian District, Beijing 100193, China. Tel.: + 86 10 62899733; fax: + 86 10 62896313.
E-mail address: yhliao@implad.ac.cn (Y.-H. Liao).

0939-6411/$ - see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2008.07.004

great portion of inhaled drug particles inevitably deposit in the conducting airway, for example, Newman and colleagues found that although aerosol administration of ciclesonide via HFA-MDI resulted in high pulmonary deposition (52% in the whole lung) in a clinical study as determined by means of three-dimensional single photon emission computed tomography, over 40% of the inhaled drug deposited in the conducting airway [13]. Drug particles depositing in the central and intermediate airway zones are subjected to mucocilliary clearance, which may lead to incomplete pulmonary absorption. Therefore, it is conceivable that retarding the mucocilliary clearance may be of significance for systemic pulmonary delivery. As a result, it is hypothesized that absorption in the conducting airway is not trivial for systemic pulmonary delivery and incorporation of mucoadhesive excipients into inhaled drug particles may prolong the airway retention time and therefore markedly enhance pulmonary bioavailability. The objectives of this study were to investigate the permeability of upper airway epithelium to scutellarin, a hydrophilic and ionized compound in neutral pH conditions, and to examine the effects of mucoadhesive excipients, which were co-spray dried with scutellarin, on the mucociliary transport and pulmonary absorption of the drug. It was also attempted to determine the applicability of inhalation delivery to achieve a high bioavailability of scutellarin in this work. The mucoadhesive excipients utilized in this study included poly(vinyl alcohol) (PVA), poly(vinyl pyrrolidone) (PVP) and hyaluronan (HA), which have been utilized in several inhalation works to improve the physical stability of drug particles in HFA propellants [14–16].

2. Materials and methods

2.1. Materials

Scutellarin was obtained by purifying commercial breviscapine (pharmaceutical grade, Wangzilong Ltd. Yunnan, China) using Sephadex LH-20 (Amersham Biosciences Ltd., Hong Kong, China) column chromatography, and its HPLC purity was determined to be more than 98%. Poly(vinyl alcohol) 80 (PVA80), poly(vinyl pyrrolidone) K15 (PVP15), pentobarbital sodium and baicalin were purchased from Sigma–Aldrich (China), sodium hyaluronate of injection grade was obtained from Tonicrays (Zhenjiang, China) and all other reagents were of analytical grade or HPLC grade and are commercially available.

2.2. Preparation of drug solutions and drug particles

Scutellarin was dissolved in a phosphate buffer (0.1 M, pH 7.3) for peroral, intratracheal and intravenous administration, respectively.

The scutellarin particles (Table 1) were prepared by spray-drying using a Model 191 Büchi mini spray-dryer following the preparation of feed solution. The processing parameters comprised a feed rate of 3 ml/min, an atomising air-flow rate of 600 L/h and an inlet temperature of 140 °C. Outlet temperatures were around 98 °C. Prior to spray-drying, scutellarin was dissolved in a phosphate buffer (0.1 M, pH 7.3), whilst polymer solutions were prepared by hydrating polymers in purified water, and subsequently, the feed solutions were obtained by mixing the drug and polymer solutions (purified water only for spray-dried scutellarin) to give a drug concentration of 4 mg/ml. Spray-dried particles were obtained in the collection jar of the spray-dryer.

Table 1
Composition of the spray-dried scutellarin particles

Spray-dried particles	Scutellarin (g)	PVP15 (g)	PVA80 (g)	HA (g)
SD-S	1.0	0	0	0
SD-SP_{15}	0.9	0.1	0	0
SD-SP_{80}	0.9	0	0.1	0
SD-$SP_{15}P_{80}$	0.8	0.1	0.1	0
SD-SH	0.9	0	0	0.1
SD-$SP_{15}P_{80}H$	0.7	0.1	0.1	0.1

2.3. Particle morphology and size analysis

Particle size and morphology were investigated using scanning electron microscopy. Powder samples were mounted onto metal sample plates and coated with gold (3 nm thick). The samples were then examined under a Jeol JSM 6000F scanning electron microscope (Tokyo, Japan), operating at 2–3 kV.

Particle size analysis was carried out using a Mastersizer 2000 laser diffraction analyser (Malvern Instruments, UK). Several milligrams of the spray-dried powders were dispersed in 1 ml of 0.1% w/v lecithin cyclohexane solution. The particle suspension was sonicated in a water bath for 30 s to disperse any possible agglomerates before being added to the sample cell. Particle size distributions were expressed in terms of volume median diameter (VMD) and span. The VMD was the diameter at the 50% point of the entire volume distribution whilst the span was defined as $[D(v, 90) - D(v, 10)]/D(v, 50)$, where $D(v, 90)$, $D(v, 50)$, and $D(v, 10)$ were the respective diameters at 90%, 50% and 10% cumulative volumes.

2.4. Aerodynamic assessment of fine particles

The aerodynamic assessment of fine particles was performed using a next generation pharmaceutical impactor (NGI) (MSP Corporation, Minneapolis, USA) after the particles were dispersed using a dry powder insufflator (Model DP-4, Penn-Century Inc., USA) following the protocol provided by the producer. Whereas intratracheal administered scutellarin solutions were delivered using a micro-sprayer (Model IA-1B, Penn-Century Inc., USA) and the aerodynamic assessment of atomized droplets was carried out by placing the tip of the sprayer in position at the end of the induction port of NGI before scutellarin solutions were atomized into the NGI, the measurements were carried out according to the method listed in the British Pharmacopoeia 2007 (Appendix XII F. Aerodynamic assessment of fine particles – fine particle dose and particle size distribution) using a flow rate of 30 L/min.

Following the recovery of the drug solution from each collection cup, the amount of the active ingredient in the cups was determined using an HPLC assay. The fine particle dose was calculated as the recovered dose of active ingredient exhibiting an aerodynamic diameter of <5 μm. The FPF was defined as the fine particle dose divided by the whole dose of the drug found in the impactor (including the throat). The recovery of drug found in the impactor relative to the emitted dose varied from 89.2% to 96.3%. The Mass Median Aerodynamic Diameter (MMAD) was the diameter at the 50% cumulative percentage whilst the geometric standard deviation (GSD) was defined as the ratio of the diameter at the 84.1% cumulative percentage to the 50%.

2.5. Analysis of the samples by HPLC

The amount of scutellarin in the samples collected in the cups of the NGI was determined using a Waters HPLC system, which included a Waters 717 plus autosampler, Waters 2487 Dual λ Absorbance Detector, Waters 600 Controller pump and an Empower software system. Each sample was injected onto a C_{18} Apollo column (150 mm × 4.6 mm, 5 μm, Alltech Associates Inc.) equipped with a Phenomenex guard column (Phenomenex Inc.) (4 mm × 3 mm). Each drug solution was analysed with an aliquot of 10 μL being applied to the column maintained at 40 °C, eluted at a flow rate 1 ml/min and detected at a wavelength of 335 nm.

An isocratic mobile phase system consisting of acetonitrile and 0.1% acetic acid aqueous solution (17:83) was employed for assaying. Before the measurements, the method was validated in terms of specificity, linearity, precision, recovery, limit of detection (LOD) and limit of quantification (LOQ), and calibration curves were determined for peak area against drug concentration of standard solutions (five concentrations between 1.0 and 50.0 μg/ml) using six replicate samples for each concentration.

2.6. In vitro mucociliary transport rate (MTR)

MTR was evaluated using a previously reported frog palate model with a slight modification [17] and calculated by dividing the distance traveled (5–10 mm) by the elapsed time (min). Briefly, spray-dried particles and graphite particles (50–100 μm in diameter) were mixed, hydrated and placed on the epithelial surface of the frog palate using a stereoscopic microscope equipped with a reticulated eyepiece, and the elapsed time for graphite particles to travel 5–10 mm was recorded. The frog palates utilized were obtained by decapitating mature frogs (*Bufo Gargarizans*, purchased from Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences, Beijing, China) immediately before the experiment. Six measurements were carried out for each tested sample. During the measurements, the frog palate was kept inside a plastic chamber with a microenvironment of approximately 100% humidity provided by nebulization of saline solution, and the experiments were carried out at room temperature (about 22 °C).

2.7. Pharmacokinetic studies

2.7.1. Animals

Animal studies were performed in compliance with protocols required by the Animal Care and Use Committee of the Chinese Academy of Medical Sciences. SPF male Wister rats (180–220 g) were procured from Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences (Beijing, China). Prior to experiments, animals, weighing 220–310 g, were acclimated for at least 7 days by breeding at specific pathogen-free (SPF) animal house and on the day before the study, a polyethylene catheter (Portex, UK) was inserted into the right jugular vein of each rat under anesthesia [18]. All animals were maintained at controlled temperature (22 ± 2 °C), under 12 h light/dark cycles, and given diet and water *ad libitum*.

2.7.2. Drug administration

Five groups of rats (each group had at least 6 rats) were administered with scutellarin solutions via peroral (dose, 50 mg/kg), intravenous (dose, 10 mg/kg) or intratracheal (dose, 2, 6 or 10 mg/kg) routes and other three groups with SD-S, SD-$SP_{15}P_{80}$, or SD-$SP_{15}P_{80}$H particles at a dose of 10 mg/kg via intrapulmonary routes after at least 24 h of recovery from intubation into the right jugular vein and 12 h of fasting. Except peroral dosing, animals were anesthetized by an intraperitoneal injection of pentobarbital sodium (40 mg/kg) prior to drug administration. For intratracheal solution administration, intratracheal spray was conducted at a volume of 1000 μl/kg after the tip of the micro-sprayer (Model IA-1B, Penn-Century Inc., USA) attached to a syringe was inserted into the trachea under visual guidance following anesthesia. For intrapulmonary particle dosing, the tip of the dry powder insufflator (Model DP-4, Penn-Century Inc., USA), loaded with scutellarin particles and attached to a syringe, was inserted into the trachea of an anesthetized rat under visual guidance, and subsequently scutellarin particles were blown into the trachea using the syringe. After administration, the micro-sprayer or insufflator was removed and the animal was held in an upright position for 1 min to ensure deposition of the dose. Intravenous dosing was carried out by injecting the drug solution (10 mg/ml) though the polyethylene catheter into the right jugular vein. However, animals subject to oral administration were not anesthetized and scutellarin solution (10 mg/ml) was administered using a 22-gauge oral dosing needle.

2.7.3. Blood sampling and sample preparation

At predetermined time points (5, 15, 30, 45, 60, 120, 180, 240, 360 and 480 min) after dosing, approximately 150 μl of blood was collected from the pre-intubated catheter and put into tubes with heparin. Subsequently, plasma samples were prepared by centrifuging for 5 min at 5000*g*, and stored at −80 °C until LC–MS/MS analysis. During the analysis, a nominal dose of baicalin (as internal standard), 20 μl of 50% phosphoric acid and 50 μl water were added to a 50 μl of plasma sample and vortexed for 30 s. The extraction of scutellarin and baicalin was subsequently carried out by addition of 800 μl of acetyl acetate and mixing for 60 s *via* vortexing. The mixture was then centrifuged for 1 min at 10,000*g*, after which 700 μl of the organic layer was transferred to a clean tube and evaporated to dryness using a nitrogen evaporator (HGC-12, Tianjin Hengao Ltd., China). For LC–MS/MS sample loading, 200 μl of water: acetonitrile (80:20, v/v) was used to reconstitute the residue, and an aliquot of 20 μl was injected onto a C8 reverse-phase column for analysis, as described below.

2.7.4. Quantification of scutellarin in plasma

Scutellarin was determined in rat plasma using a sensitive and selective LC–MS/MS method, which was performed on a 3200Qtrap Mass Spectrometer (Applied Biosystems Inc., USA) equipped with an electrospray ionization (ESI) source system and an Agilent 1200 HPLC system (Wilmington, DE) consisting of a vacuum degasser, a binary pump, an autosampler, and an Analysis software system. Each sample was injected onto an XTerra MS C8 Waters column (50 mm × 2.1 mm, 3 μm). The mobile phase was composed of 1% (v/v) formic acid in water and acetonitrile (80:20, v/v). The flow rate was set at 0.3 ml/min with a run time of 4 min. The column was maintained at 40 °C. The LCQ interface was adjusted to the following conditions: ion mode, negative; spray voltage, -4.5 kV; capillary temperature, 350 °C; gas 1 (nitrogen), 60 L/h; and gas 2 (nitrogen), 40 L/h. Multitude reaction monitoring included m/z 461.0–m/z 285.0 for scutellarin and the metabolite and m/z 445.0–m/z 269.0 for baicalin.

Stock solution of scutellarin and baicalin (used as internal standard) was prepared by dissolving the appropriate amount of powder in methanol to yield the concentration of 0.1 mg/ml. Working solutions of scutellarin were prepared by appropriate dilution whilst the concentration of internal standard was maintained at 500 ng/ml. All these solutions were stored at −20 °C for less than 14 days.

The method was validated in terms of linearity, precision, recovery, limit of detection (LOD), limit of quantification (LOQ) and robustness to "fit the purpose" of analysis in this study. The method provided good linearity ($r^2 \geqslant 0.9999$) over a concentration range of 1–5000 ng/ml, and the standard calibration curves for scutellarin were constructed using the analyte/internal standard peak-area ratios versus the nominal concentrations of the analyte. The precision of each assay was evaluated by determining the intra-run and inter-run relative standard deviation (RSD) of six different concentrations of the standard solutions. The intra- and inter-precision were found to be less than 3.0% and 5.0%, respectively.

The recovery of scutellarin was determined as follows: scutellarin and baicalin were spiked to blank plasma samples to give scutellarin concentrations of 1, 10, 100, 500, 1000 and 5000 ng/ml and maintain the concentration of baicalin at 500 ng/ml, and assayed using the method described above. There was not any detectable amount of the test compound recovered in blank plasma samples,

and recovery was calculated by comparing the peak area of the extracted sample (corrected according to the volume transferred during extraction) to that of the unextracted standard solution containing the same concentration with recovery being not less than 80%. The LOD and LOQ were calculated from the equations $LOD = 3 \times S_{blank}$ and $LOQ = 10 \times S_{blank}$ with value being 0.3 and 1 ng/ml respectively.

The robustness tested the stability of standard solutions and the effect of extraction solvent (type and volume) and extraction procedure (mixing time) on the drug stability and recovery. The results showed that standard solutions were stable for at least 24 h at room temperature in brown volumetric flasks. The extraction protocol utilized did not significantly affect the drug stability and recovery.

2.8. Culture of A549 and Calu-3 cell lines and MTT assay

The Calu-3 and A549 cell lines were obtained from the Cell Culture Center, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences (Beijing, China) and used between passages 38–47 and 42–50, respectively. Cell cultures were grown using 75 cm^2 flasks in a humidified 5% CO_2/95% atmospheric air incubator at 37 °C. For Calu-3 cells, cell culture medium (CCM) was 500 ml Eagle's Minimum Essential Medium, 50 ml foetal bovine serum, 5 ml non-essential amino acid solution, 5 ml L-glutamine solution (200 mM) and 0.5 ml Penicillin–Streptomycin. For the A549 cells, 500 ml F-12 Ham, 50 ml foetal bovine serum, 5 ml L-glutamine solution (200 mM) and 0.5 ml Penicillin–Streptomycin were used. Medium was exchanged every 2–3 days and cells were subcultured weekly. For the MTT assay, Calu-3 and A549 cells were seeded at a density of 1×10^4 cells/well in 96-well plates in 100 μl of the same medium used for culture in cell culture flasks. The cells were grown at 37 °C in a 5% CO_2 atmosphere for 24 h before use in cell viability assays.

PVA80, PVP K15, HA, and SD-S, SD-$SP_{15}P_{80}$ and SD-$SP_{15}P_{80}$H particles were assayed for cytotoxicity over 24 and 48 h on Calu-3 and A549 cell lines, with sodium dodecyl sulfate (SDS, 2%) as a positive control based on a previous reported protocol [19]. Optimal cell seeding density and the absence of MTT conversion in assay medium was confirmed in preliminary experiments. PVA80, PVP K30 and HA were hydrated overnight whilst the spray-dried particles were dissolved in DMSO, and consequently, all tested samples and controls were prepared as solution in pre-warmed CCM with 2% FBS immediately before application to the cells. To initiate the assay, culture medium of A549 and Calu-3 cells at 24 h in culture was replaced by 100 μl of fresh medium containing the test solutions or controls at a range of predetermined concentrations. After 24 or 48 h of cell incubation with the samples, 20 μl of the MTT solution (0.5 mg/ml in PBS, pH 7.3) was added to each well. After 4 h, medium was removed and any formazan crystals generated were solubilised with 200 μl DMSO. Upon complete solubilisation of the crystals, the absorbance of each well was measured by spectrophotometry (μQuant™, BioTek Instruments, Inc., USA) at 570 nm and corrected for background absorbance using a wavelength of 650 nm.

2.9. Data analysis

The values of area under the plasma concentration–time curve (AUC) were calculated using the trapezoidal rule, whilst the C_{max} and T_{max} values were read directly from the concentration–time profile. All AUC values were calculated for each individual animal or group of animals before determining mean values, and the absolute bioavailability (F) was calculated by Eq. (1). Statistical analysis was carried out using unpaired Student's t-test (SPSS 11.0 for windows), and data are presented as means ± SD, unless otherwise stated.

$$F = \frac{\mathrm{AUC/mass}}{\mathrm{AUC_{iv}/mass_{iv}}} \times 100\% \quad (1)$$

3. Results and discussion

3.1. Pharmacokinetic profiles of scutellarin solution administrated by different routes

Flavonoids are a very important type of effective ingredients present generally as glycosylated forms in traditional Chinese medicine. Scutellarin is a flavonoid glucuronide isolated from plants; it has been demonstrated that the drug undergoes dramatic first-pass metabolism after oral administration. Earlier studies reported that at first orally delivered scutellarin is most likely metabolized to its aglycone, subsequently penetrates through intestinal membrane, and the absorbed aglycone molecules are further metabolized to conjugated forms such as glucuronides, sulfates, or methylated derivatives [2]. However, the major components present in plasma are its parent drug and a 6-*O*-glucuronide metabolite, isoscutellarin after peroral administration [2]. In this study, the major components present in rat plasma were also found to be scutellarin and isoscutellarin after peroral administration as shown in multiple reaction monitoring (m/z 461–m/z 285) LC–MS/MS chromatograms (data not shown). In contrast, the peak assigned to isoscutellarin in LC–MS/MS chromatograms (data not shown) was not found in rat plasma samples when scutellarin was administered either intravenously or intratracheally. The absorption and metabolism of flavonoids in the respiratory tract has not been documented previously, and findings in this study strongly suggested that scutellarin is most likely absorbed as its parent form, avoiding the first-pass metabolism occurring in the gastrointestinal tract.

The absorption profiles of scutellarin from different administered routes in rats to the systemic circulation were found to be different. Fig. 1a shows the concentrations of scutellarin in plasma as a function of time after administration via three delivery routes. The corresponding AUC_{0-480}, absolute bioavailability, C_{max}, and t_{max} parameters are summarized in Table 2. Like intravenous injection, intratracheal delivery produced the peak plasma concentration of scutellarin within 5 min, which was greatly shorter than the t_{max} of peroral route. Whereas the peroral route of administration provided an irregular plot of plasma concentration vs time and gave the C_{max} more than 200 min, which were in agreement with previous results [20]. In addition, the absolute bioavailability in terms of AUC_{0-480} of intratracheal spray of scutellarin was found to be 77.2 ± 3.7%, which was significantly ($p < 0.001$) higher than that of peroral delivered drug (2.3 ± 0.54%). In this study, the drug solution was intratracheally delivered using a micro-sprayer which produced droplets with MMAD being larger than 11.7 μm (Fig. 2), and hence the droplets should mainly deposit in the upper respiratory tract. As a consequence, the high pulmonary bioavailability indicated that the conducting airway epithelium is well permeable to scutellarin.

Moreover, the AUC were found to increase linearly with delivered dose when scutellarin solution was administered via intratracheal route at three doses of 2.0, 6.0 and 10.0 mg/kg. The AUC_{0-480} of scutellarin administered at the three doses were found to be 22.1 ± 2.8, 81.3 ± 6.3 or 145.7 ± 6.4 μg/ml min (Table 2.), respectively. Although the bioavailability at the dose of 2.0 mg/kg seemed to be significantly ($p < 0.001$) lower than that at 10 mg/kg, the plots for AUC *vs* dose taken provided a high degree of linearity ($r^2 = 0.997$). The reason for lower bioavailability at low dose is

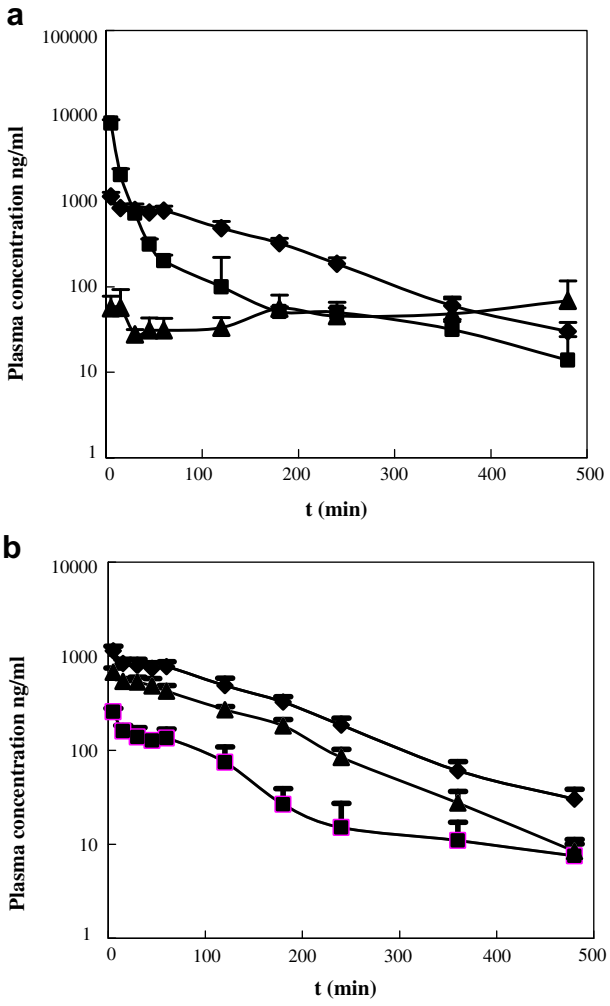

Fig. 1. Means (±SEM, $n = 6$) plasma concentration in plasma as a function of time (a) following intravenous (square, dose at 10.0 mg/kg), intratracheal (diamond, dose at 10.0 mg/kg), or peroral (triangle, dose at 50.0 mg/kg) administrations of scutellarin solution to rats; (b) following intratracheal administrations of scutellarin solution at doses of 2.0 mg/kg (square), 6 mg/kg (triangle) or 10 (diamond) mg/kg to rats.

Table 2
Pharmacokinetic parameters in plasma after intravenous (IV, dose at 10 mg/kg), intratracheal (IT, dose at 2, 6, 10 mg/kg) or peroral (PO, dose at 50 mg/kg) administrations of scutellarin solution to rats, or after intrapulmonary insufflation of spray-dried particles (SD, dose at 10 mg/kg)

	AUC_{0-480} (μg/ml min)	Absolute bioavailability (%)	C_{max} (ng/ml)	T_{max} (min)
$IV_{10\,mg/kg}$	188.6 ± 9.4	100	>8230 ± 790	<5.0
$PO_{50\,mg/kg}$	21.85 ± 4.3	2.3 ± 0.54	<100	>200
$IT_{10\,mg/kg}$	145.7 ± 6.4	77.2 ± 3.7	>1140 ± 140	<5.0
$IT_{6\,mg/kg}$	81.3 ± 6.3	71.4 ± 5.6	>678 ± 71	<5.0
$IT_{2\,mg/kg}$	22.1 ± 2.8	58.5 ± 8.4	>257 ± 21	<5.0
SD-S	132.3 ± 7.7	70.1 ± 4.0	>5070 ± 530	<5.0
$SD\text{-}SP_{15}P_{80}$	165.3 ± 13.4	87.6 ± 7.1	>5970 ± 650	<5.0
$SD\text{-}SP_{15}P_{80}H$	184.7 ± 14.9	97.9 ± 7.9	>3650 ± 550	<5.0

not clear but might be related to a saturable pre-absorptive first-pass metabolism in the respiratory tract. Indeed, co-incubation of scutellarin and rat bronchial alveolar lavage in a phosphate buffer saline (pH 7.4) led to a minor decrease in the content of the drug within 30 min, but further increase in incubation time did not result in further detectable degradation (data not shown). In addition, the mean plasma concentration–time profiles of scutellarin

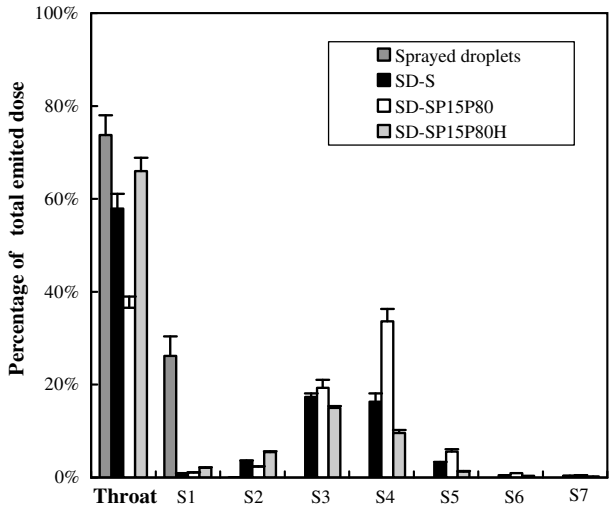

Fig. 2. Particle mass distributions of spray-dried scutellarin particles as dispersed using a dry powder insufflator or sprayed scutellarin droplets generated by a Micro-Sprayer as measured by Next Generation Impactor. The cut off diameters of $S1 = 11.71$ μm, $S2 = 6.40$ μm, $S3 = 3.99$ μm, $S4 = 2.30$ μm, $S5 = 1.36$ μm, $S6 = 0.83$ μm, $S7 = 0.54$ μm, data presented as means ± SD, $n = 3$.

at three doses showed the same t_{max} and exhibited similar curve shapes, suggesting similar pulmonary absorption kinetics (Fig. 1b).

Therefore, based upon comparative pharmacokinetic studies of scutellarin in plasma after intratracheal, intravenous and peroral routes of administration to rats, it could be concluded that pulmonary delivery of scutellarin could eliminate the first-pass metabolism associated with peroral drug delivery and confer a high absolute bioavailability.

3.2. Particle size, morphology and aerodynamic characteristics of spray-dried particles

Various techniques (e.g. spray-drying) have been utilized for the preparation of micron-size particles suitable for pulmonary drug delivery [21,22]. In this study, respirable drug containing particles were prepared by spray-drying. The morphology of spray-dried particles was examined using scanning electron microscopy (SEM). SEM results showed that the surface morphology of spray-dried particles could vary from smooth to moderately dimpled, and to raisin-like depending upon spray-drying parameters and preparation compositions, for example, increasing inlet temperature, decreasing atomising air-flow rate or feed rate and/or adding PVA80 to the feed solution tended to increase the corrugated degree of particle surface (data not shown). In addition, pulmonary delivery of spray-dried particles was carried out using a dry powder insufflator and $SD\text{-}SP_{15}$, $SD\text{-}SP_{80}$ and SD-SH appeared not to be well dispersible via the insufflator, which provided irregular emitted doses; as a consequence, no further particle characterization and *in vivo* delivery were conducted for those particles. The three batches of spray-dried particles tested in this study, namely SD-S, $SD\text{-}SP_{15}P_{80}$, and $SD\text{-}SP_{15}P_{80}H$, respectively, were found to possess a similar surface morphology, presenting smooth surfaces with multiple dimples (data not shown).

The particle size and size distribution of the spray-dried particles were determined using both a laser diffraction analyser and a NGI, and the results are shown in Table 3. The volume median diameters (VMDs) of three batches of spray-dried particles were found to be between 2.11 and 2.48 μm with the particle size span lying between 1.52 and 1.59, which indicates that the three batches of particles exhibited a similar geometric size distribution of characteristics.

Table 3
Particle size, size distribution and fine particle fraction of spray-dried particles (means ± SD, $n \geqslant 3$)

	VMD (μm)	Span	MMAD (μm)	GSD	FPF (%)
SD-S	2.11 ± 0.03	1.52 ± 0.01	2.38 ± 0.12	1.53 ± 0.02	39.4 ± 2.9
SD-$SP_{15}P_{80}$	2.42 ± 0.02	1.59 ± 0.01	1.95 ± 0.09	1.61 ± 0.01	61.1 ± 4.9
SD-$SP_{15}P_{80}$H	2.48 ± 0.05	1.57 ± 0.01	2.83 ± 0.15	1.58 ± 0.05	29.1 ± 1.2

The aerodynamic mass distribution data for spray-dried scutellarin particles after being dispersed using a dry powder insufflator from NGI are shown in Fig. 2. The fine particle fractions (FPF) (<5 μm) generated by SD-S, SD-$SP_{15}P_{80}$ and SD-$SP_{15}P_{80}$H particles were found to be 39.4%, 61.1% and 29.1%, respectively. The mass median aerodynamic diameters (MMADs) for three batches of the particles were 2.38, 1.95 and 2.83, respectively (Table 3). Although particle size distribution data from NGI are intended for simulation of likely performance in clinical use, such data should also be applicable for simulation of rat lung deposition efficiency of particles since the relationship between particle size and deposition in the central and peripheral airways is similar between human and rats [36].

3.3. In vitro mucociliary transport

In vitro mucociliary transport results obtained from a frog palate model showed that the transport rates of graphite particles (MTR) on frog palate mucus were highly dependent upon the presence of mucoadhesive excipients in spray-dried particles (Fig. 3). When suspended graphite particles in saline were placed on the mucus of a frog palate, the MTR was found to be about 17.5 mm/min and it appeared not to be affected after a palate had been exposed to mucoadhesive polymers for 10 min or repeatedly used for the MTR measurement of spray-dried particles (data not shown). As a result, it was assumed that polymers and spray-dried particles had no toxic effect on the palate mucus. In addition, mixing graphite particles with spray-dried scutellarin particles appeared not to significantly affect the MTR ($p > 0.05$). However, a significant decrease in MTR was observed when graphite particles were mixed with co-spray-dried scutellarin-polymer(s) particles, for example, the MTR was found to decrease by about threefold and sixfold when mixed with SD-$SP_{15}P_{80}$, and SD-$SP_{15}P_{80}$H particles, respectively. Decrease in MTR in the presence of hydrated spray-dried particles might result from the mucoadhesion of PVA 80 and/or HA, and the mechanism by which the polymers adhered to mucus might be related to the formation of hydrogen bonding interactions between hydroxyl or carboxyl groups in polymer molecules and mucus molecules [37].

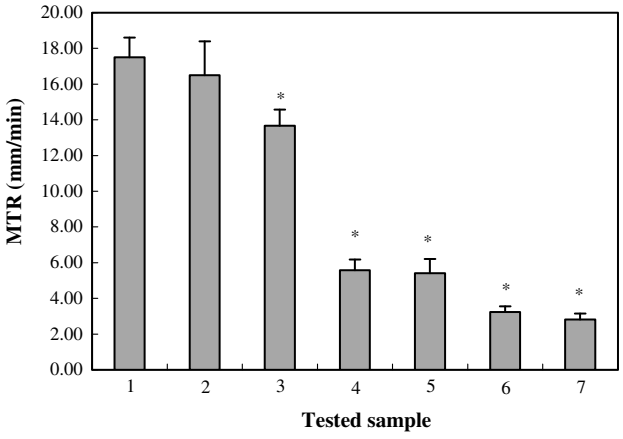

Fig. 3. The mucociliary transport rates of graphite particles when mixed with co-spray-dried scutellarin and mucoadhesive excipients particles tested using a frog palate model (means ± SD, $n = 6$). Sample 1, graphite particles alone; Sample 2, with SD-S; Sample 3, with SD-SP_{15}; Sample 4, with SD-SP_{80}; Sample 5, with SD-$SP_{15}P_{80}$; Sample 6, with SD-SH; Sample 7, with SD-$SP_{15}P_{80}$H. (*significantly different compared to sample 1, $p < 0.001$).

3.4. Pulmonary absorption of dispersed spray-dried scutellarin containing particles

Pharmacokinetic profiles of SD-S, SD-$SP_{15}P_{80}$, and SD-$SP_{15}P_{80}$H particles following intrapulmonary administrations to rats were also investigated. The pulmonary absorption profiles of the particles are shown in Fig. 4, and the corresponding pharmacokinetic parameters are listed in Table 2. The results showed that compared to intratracheally delivered scutellarin solution (Fig. 1b), intrapulmonary administrations of spray-dried scutellarin containing particles led to a more rapid initial absorption rate (Fig. 4) with C_{max} ranging from 3650 ± 550 to 5970 ± 650 ng/ml, significantly ($p < 0.001$) higher than that of the former (1140 ± 138 ng/ml) when the administered dose was maintained at 10 mg/kg. Such differences in C_{max} might be attributable to the fact that dispersed spray-dried scutellarin particles were more efficiently deposited in the peripheral lung and hence more extensively absorbed within 5 min than sprayed droplets of scutellarin solution (Fig. 2). Differences in the FPF also accounted for the differences in the C_{max} of the three batches of spray-dried particles, amongst which, SD-$SP_{15}P_{80}$ particles possessed the highest FPF and resulted in the largest C_{max} of 5970 ± 650 ng/ml whereas SD-$SP_{15}P_{80}$H particles had the lowest FPF and gave the smallest C_{max} of 3650 ± 550 ng/ml, which was significantly ($p < 0.001$) smaller than that of SD-$SP_{15}P_{80}$.

However, the magnitude of AUC_{0-480} of intratracheally delivered particles was not found to be correlated to the FPF. For example, although SD-$SP_{15}P_{80}$H particles had the lowest FPF, the AUC_{0-480} appeared to be the highest amongst the three batches of particles. The AUC_{0-480} of the three batches of particles were determined to be 132.3 ± 7.7, 165.3 ± 13.4 and 184.7 ± 14.9 μg/ml·min, respectively, corresponding to the absolute bioavailability of 70.1 ± 4.0%, 87.6 ± 7.1% and 97.9 ± 7.9%, respectively (Table 2). The bioavailability resulting from the three batches of particles was inversely related to the corresponding mucociliary transport rate (MTR). The reason for such a relationship might be attributable to the assump-

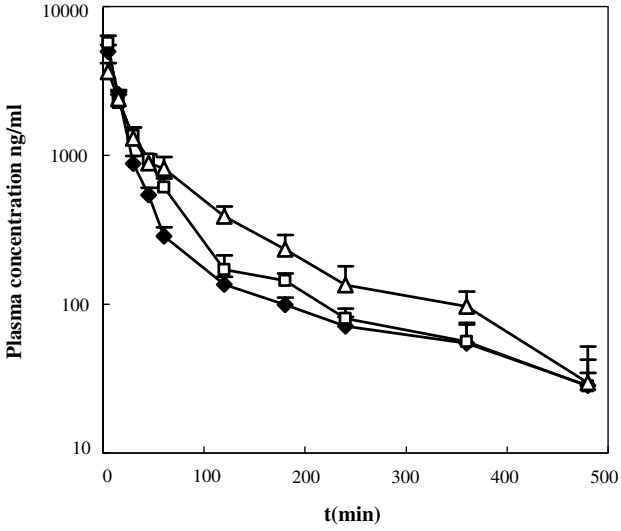

Fig. 4. Means (±SEM, $n = 6$) plasma concentration of scutellarin as a function of time following intrapulmonary administrations of spray-dried particles at a drug dose of 10 mg/kg to rats, SD-S (diamond), SD-$SP_{15}P_{80}$ (square) and SD-$SP_{15}P_{80}$H (triangle).

Table 4
A549 and Calu-3 cell viability measured by MTT cytotoxicity assay after 24 and 48 h exposure to spray-dried scutellarin particle solutions

Test samples	Test concentration mg/ml	A549 cell viability at 24 h (%)	A549 cell viability at 48 h (%)	Calu-3 cell viability at 24 h (%)	Calu-3 cell viability at 48 h (%)
SD-S	0.05	103.5 ± 10.7	102.4 ± 8.9	92.3 ± 13.5	91.2 ± 1.7
SD-S	0.5	123.0 ± 7.5	121.1 ± 4.7	110.4 ± 13.0	93.7 ± 11.8
SD-S	5.0	92.9 ± 6.8	88.9 ± 7.8	125.2 ± 21.4	103.0 ± 9.1
SD-$SP_{15}P_{80}$	0.05	93.5 ± 8.1	84.9 ± 6.1	88.2 ± 7.8	98.4 ± 3.2
SD-$SP_{15}P_{80}$	0.5	123.0 ± 8.4	108.1 ± 9.4	99.3 ± 11.8	89.6 ± 7.2
SD-$SP_{15}P_{80}$	5.0	91.4 ± 6.2	101.1 ± 5.3	106.1 ± 11.4	107.5 ± 7.8
SD-$SP_{15}P_{80}H$	0.05	100.1 ± 4.8	93.5 ± 7.7	83.4 ± 2.9	79.2 ± 1.3
SD-$SP_{15}P_{80}H$	0.5	103.0 ± 8.4	109.7 ± 8.6	89.5 ± 1.7	79.7 ± 7.8
SD-$SP_{15}P_{80}H$	5.0	101.1 ± 8.8	101.1 ± 4.7	116.3 ± 10.7	110.4 ± 6.8

Data represent means ± SD (n = 6) from one of three experiments.

tion that a complete pulmonary absorption could be achieved, only if the drug particles depositing in the upper respiratory tract are retained long enough to escape from mucociliary clearance. Indeed, for those intrapulmonary delivered particles, a considerable portion of drug deposits in the upper respiratory tract, where the drug is subjected to mucociliary clearance, and the absorption of this part of drug dictate the extent of pulmonary drug absorption. Previously, studies had demonstrated that incorporating a mucoadhesive, hydroxypropyl cellulose (HPC), into inhaled drug microspheres conferred retardation of the mucociliary clearance, and as a consequence, resulted in enhanced pulmonary absorption of delivered drugs [23,24]. In this study, the presence of mucus adhesive excipients such as PVA80 and HA in spray-dried particles led to a decrease in MTR and an increase in the mucus retention capacity such that the pulmonary absorption of the drug was extended to a relatively long time, leading to high bioavailability. For successful development of a new aerosol delivery system for systemic pulmonary delivery, enhancing the absorption of drug molecules, particularly those with slow absorption rates in the airway epithelium, depositing in the conducting airways, might not be trivial. Although novel particle engineering technology and devices may achieve a highly efficient total lung deposition (TLD), over 60% of TLD is in the central and intermediate airway zones [25]. Incorporation of bioadhesive excipients into the respirable particles may prolong the retention time of drug particles and as a consequence, lead to a more extensive pulmonary absorption.

3.5. Pulmonary biocompatibility of spray-dried scutellarin particles

Delivery of flavonoids containing traditional Chinese medicine (TCM) to the respiratory tract via nebulizers has been widely utilized in clinic in China for the management of various pulmonary diseases and infections, however, it should be noted that these medicines were initially approved for injection rather than inhalation therapy, and their pulmonary toxicity profiles have yet been thoroughly established. Nonetheless, various clinical studies suggested that inhaled flavonoids might be safe without pulmonary side effects being clinically observed, for example, over 100 clinical papers have acknowledged the safety and efficacy of nebulized Shuang-Huang-Lian, a famous compound TCM formula containing baicalin, a flavonoid glucuronide analogue of scutellarin, as one of the main constituents [26]. In this study, the potential pulmonary toxicity of spray-dried scutellarin particles was *in vitro* evaluated using two lung epithelial cell lines, Calu-3 and A549, which had been previously utilized for the evaluation of airway and alveolar toxicities [27–29]. The results showed that the three spray-dried scutellarin particles exhibited no local cell cytotoxic effects in the tested concentrations ranging from 0.05 to 5 mg/ml (Table 4), and hence might be biocompatible to the lung.

The choice of bioadhesives for inhalation delivery will depend on not only the mucoadhesive capacity but also the pulmonary biocompatibility. Although various polymer bioadhesives (e.g., PVA [14,15], HPC [23,24], chitosans [30,31], hyaluronan [32,33], etc.) have been reported in pulmonary delivery studies, no mucoadhesive excipient has been approved for inhalation purpose up to date. However, a previous study suggested that PVA (0.1–10 mg/ml) and hyaluronan (0.1–5 mg/ml) appeared not to be toxic over the concentration ranges used on both 16HBE14o- and A549 cell lines, whilst chitosan at high concentration was toxic on 16HBE14o- cell line, albeit not on A549 cell line [34]. Results in this study also showed that PVA, PVP and HA were not toxic to both Calu-3 and A549 cell lines (Data not shown). Considering its mucoadhesive ability and pulmonary endogeneity [35], hyaluronan may have the highest potential to be a mucoadhesive for inhalation delivery.

4. Conclusions

Pharmacokinetic results from this study suggest that scutellarin is more rapidly and thoroughly absorbed following intratracheal administration to the rat lung, as compared to peroral administration. In addition, pulmonary delivery of scutellarin seemed to eliminate the intestinal and hepatic first-pass metabolism occurring during oral delivery of the drug with the absolute pulmonary bioavailability at the dose of 10 mg/kg against intravenous controls being determined to be about 77%, which was more than 30-fold higher than that given by peroral administration. Further results indicate that the initial pulmonary absorption rates of scutellarin were positively related to the respirable fraction of intrapulmonary delivered particles or droplets since drug depositing in the peripheral lung is more readily absorbed than that in the upper region, whereas the extent of absorption was dependent upon the presence of mucoadhesive excipients in the delivered particles. Indeed, with decreasing the MTR of spray-dried scutellarin particles by about sixfold as a result of adding mucoadhesive polymeric mixtures to the particles, the absolute bioavailability of the drug was found to increase from 70.1% to 97.9%. Moreover, spray-dried scutellarin particles appeared to be biocompatible to the respiratory tract. Therefore, these findings suggest that prolonging the absorption of drug depositing in the conducting airway as a result of incorporating mucoadhesives conferred significantly improved systemic bioavailability, and pulmonary delivery may be a promising and an effective non-invasive route for the systemic delivery of poorly orally absorbed hydrophilic glycosylated flavonoids like scutellarin.

Acknowledgments

The authors thank Dr. Ben Forbes for his kind suggestions on cell cultures, and the National Natural Science foundation of China (Grant No. 30400572), the Beijing Natural Science Foundation (Grant No. 7072046) and the National Key Technology R&D Program (Grant No. 2006BAI09B08-11) for the financial supports.

References

[1] N. Wei, Advances of breviscapine in clinical applications and its side-effects, China Pharm. 4 (2001) 144–146.
[2] X.Y. Chen, L. Cui, X.T. Duan, B. Ma, D.F. Zhong, Pharmacokinetics and metabolism of the flavonoid scutellarin in humans after a single oral administration, Drug Metab. Dispos. 34 (2006) 1345–1352.
[3] F. Xiong, H. Wang, J. Cheng, J. Zhu, Determination of scutellarin in mouse plasma and different tissues by high-performance liquid chromatography, J. Chromatogr. B 835 (2006) 114–118.
[4] W. He, F. Zeng, Effect of breviscapine on ischemic cerebrovascular disease and its clinical investigation, Chin. J. Clin. Pharmacol. 18 (2002) 458–461.
[5] H. Hong, G.Q. Liu, Protection against hydrogen peroxide-induced cytotoxicity in PC12 cells by scutellarin, Life Sci. 74 (2004) 2959–2973.
[6] H. Liu, X. Yang, R. Tang, J. Liu, H. Xu, Effect of scutellarin on nitric oxide production in early stages of neuron damage induced by hydrogen peroxide, Pharmacol. Res. 51 (2005) 205–210.
[7] X. Hao, G. Cheng, J. Yu, Y. He, F. An, J. Sun, Study on the role of hepatic first-pass elimination in the low oral bioavailability of scutellarin in rats, Pharmazie 60 (2005) 477–478.
[8] J.S. Patton, C.S. Fishburn, J.G. Weers, The lungs as a portal of entry for systemic drug delivery, Proc. Am. Thorac. Soc. 1 (2004) 338–344.
[9] R.U. Agu, M.I. Ugwoke, M. Armand, R. Kinget, N. Verbeke, The lung as a route for systemic delivery of therapeutic proteins and peptides, Respir. Res. 2 (2001) 198–209.
[10] B.L. Laube, The expanding role of aerosols in systemic drug delivery gene therapy and vaccination, Respir. Care 50 (2005) 1161–1174.
[11] J. Heyder, Deposition of inhaled particles in the human respiratory tract and consequences for regional targeting in respiratory drug delivery, Proc. Am. Thorac. Soc. 1 (2004) 315–320.
[12] P.G. Noone, J.A. Regnis, X.R. Liu, K.L.R. Brouwer, M. Robinson, L. Edwards, M.R. Knowles, Airway deposition and clearance and systemic Pharmacokinetics of amiloride following aerosolization with an ultrasonic nebulizer to normal airways, Chest 112 (1997) 1283–1290.
[13] S. Newman, A. Salmon, R. Nave, A. Drollmann, High lung deposition of 99mTc-labeled ciclesonide administered via HFA-MDI to patients with asthma, Respir. Med. 100 (2006) 375–384.
[14] S.A. Jones, G.P. Martin, M.B. Brown, Manipulation of beclomethasone–hydrofluoroalkane interactions using biocompatible macromolecules, J. Pharm. Sci. 95 (2006) 1060–1074.
[15] S.A. Jones, G.P. Martin, M.B. Brown, Stabilisation of deoxyribonuclease in hydrofluoroalkanes using miscible vinyl polymers, J. Control. Release 115 (2006) 1–8.
[16] Y.H. Liao, M.B. Brown, S.A. Jones, T. Nazir, G.P. Martin, The effects of polyvinyl alcohol on the in vitro stability and delivery of spray-dried protein particles from surfactant-free HFA 134a-based pressurised metered dose inhalers, Int. J. Pharm. 304 (2005) 29–39.
[17] S.T. Lim, G.P. Martin, D.J. Berry, M.B. Brown, Preparation and evaluation of the in vitro drug release properties and mucoadhesion of novel microspheres of hyaluronic acid and chitosan, J. Control. Release 66 (2000) 281–292.
[18] Q. Chang, Z. Zuo, M.S.S. Chow, W.K.K. Ho, Difference in absorption of the two structurally similar flavonoid glycosides hyperoside and isoquercitrin in rats, Eur. J. Pharm. Biopharm. 59 (2005) 549–555.
[19] A. Grenha, C.I. Grainger, L.A. Dailey, B. Seijo, G.P. Martin, C. Remuñán-López, B. Forbes, Chitosan nanoparticles are compatible with respiratory epithelial cells in vitro, Eur. J. Pharm. Sci. 31 (2007) 73–84.
[20] H.Y. Zhang, Q.N. Ping, J.X. Guo, F. Cao, Pharmacokinetics of breviscapine and its (-cyclodextrin complex in rats, Acta Pharm. Sinica 40 (2005) 563–567.
[21] R. Vehring, Pharmaceutical particle engineering via spray drying, Pharm. Res. 25 (2008) 999–1022.
[22] P.C. Seville, H.Y. Li, T.P. Learoyd, Spray-dried powders for pulmonary drug delivery, Crit. Rev. Ther. Drug Carrier Syst. 24 (2007) 307–360.
[23] M. Sakagami, K. Sakon, W. Kinoshita, Y. Makino, Enhanced pulmonary absorption following aerosol administration of mucoadhesive powder microspheres, J. Control. Release 77 (2001) 117–129.
[24] M. Sakagami, W. Kinoshita, K. Sakon, J. Sato, Y. Makino, Mucoadhesive beclomethasone microspheres for powder inhalation: their pharmacokinetics and pharmacodynamics evaluation, J. Control. Release 80 (2002) 207–218.
[25] O.S. Usmani, M.F. Biddiscombe, P.J. Barnes, Regional lung deposition and bronchodilator response as a function of (2-agonist particle size, Am. J. Respir. Crit. Care Med. 172 (2005) 1497–1504.
[26] C. Wang, X.B. Liu, L.H. Quan, C.Y. Liu, Y.H. Liao, Pulmonary delivery of traditional Chinese medicines, World Sci. Technol. Mod. Tradit. Chin. Med. 9 (2007) 59–64.
[27] B.I. Florea, M. Thanou, H.E. Junginger, G. Borchard, Enhancement of bronchial octreotide absorption by chitosan and *N*-trimethyl chitosan shows linear in vitro/in vivo correlation, J. Control. Release 110 (2006) 353–361.
[28] B.I. Florea, M.L. Cassara, H.E. Junginger, G. Borchard, Drug transport and metabolism characteristics of the human airway epithelial cell line Calu-3, J. Control. Release 87 (2003) 131–138.
[29] B. Forbes, C. Ehrhardt, Human respiratory epithelial cell culture for drug delivery applications, Eur. J. Pharm. Biopharm. 60 (2005) 193–205.
[30] H. Yamamoto, Y. Kuno, S. Sugimoto, H. Takeuchi, Y. Kawashima, Surface-modified PLGA nanosphere with chitosan improved pulmonary delivery of calcitonin by mucoadhesion and opening of the intercellular tight junctions, J. Control. Release 102 (2005) 373–381.
[31] M. Amidi, H.C. Pellikaan, A.H. de Boer, D.J.A. Crommelin, W.E. Hennink, W. Jiskoot, Preparation and physicochemical characterization of supercritically dried insulin-loaded microparticles for pulmonary delivery, Eur. J. Pharm. Biopharm. 68 (2008) 191–200.
[32] K. Morimoto, K. Metsugi, H. Katsumata, K. Iwanaga, M. Kakemi, Effects of low-viscosity sodium hyaluronate preparation on the pulmonary absorption of rh-insulin in rats, Drug Dev. Ind. Pharm. 27 (2001) 365–371.
[33] K. Surendrakumar, G.P. Martyn, E.C.M. Hodgers, M. Jansen, J.A. Blair, Sustained release of insulin from sodium hyaluronate based dry powder formulations after pulmonary delivery to beagle dogs, J. Control. Release 91 (2003) 385–394.
[34] A. Quader, Spray Dried Formulations for the Pulmonary Delivery of Peptides and Proteins. Ph.D. Thesis, University of London, 2004.
[35] Y.H. Liao, S.A. Jones, B. Forbes, M.B. Brown, G.P. Martin, Hyluronan: pharmaceutical characterization and drug delivery, Drug Deliv. 6 (2005) 327–342.
[36] R.B. Schlesinger, Comparative deposition of inhaled aerosols in experimental animals and humans: a review, J. Toxicol. Environ. Health 15 (1985) 197–214.
[37] J.D. Smart, The basics and underlying mechanisms of mucoadhesion, Adv. Drug Deliv. Rev. 57 (2005) 1556–1568.