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High azithromycin loading powders for inhalation and their *in vivo* evaluation in rats

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ABSTRACT

The aim of the present work is to develop high azithromycin loading powders for inhalation with optimal physical and aerodynamic properties, and to test their *in vivo* potential in rats. A five-level three-factorial central composite rotatable design was used for conducting the experiments. The optimized powder, which had a better flowability, an aerodynamic diameter of 3.82 μm and an *in vitro* deposition of 51%, was obtained under selected spray-drying conditions. The content of the powder was high (59.2%), which met the requirement for high drug loading. The optimized powder was further examined in the *in vivo* study in rats. Lung microdialysis and blood microdialysis were simultaneously performed on each rat. The ratio of the AUC_{ELF} value between the intratracheal route and intravenous injection was 161.6, whereas the absolute bioavailability was only 43.5%, and the drug targeting index of the intratracheal route was 486.2. The results showed that azithromycin dry powder inhalers (DPI) could be effectively and efficiently delivered to a specific target site and achieve a high local concentration. In conclusion, AZI DPI offers an attractive alternative that is able to deliver high concentrations of antibiotic directly to the chosen target site while minimizing the systemic bioavailability and side effects.

1. Introduction

Community-acquired pneumonia (CAP) is a common respiratory tract infection and is of growing concern to public health workers worldwide. The annual incidence rates in the US range from 2.67/1000 to 12/1000 while in European countries the lowest rates are reported from Denmark with 0.10–0.18/1000 and the highest is in Spain reaching 13.4/1000 (Kohlhammer et al., 2007). Similarly, hospital-acquired pneumonia (HAP), defined as occurring more than 48 h after hospital admission, is the second most common hospital-acquired infection and is associated with the greatest number of nosocomial related deaths (Kollef, 2005). Although the introduction of antimicrobial therapy has substantially improved the outcome of respiratory tract infections in both outpatients and critically ill patients, there are a remarkable number of cases in which antimicrobial therapy fails to be effective. This is largely due to the increasing presence of pathogenic microorganisms with resistance to existing antimicrobial agents, which results in the administration of ineffective treatments.

One possible explanation for this resistance relates to the inability of the antibiotic to achieve a high enough concentration at the target site. Although it has commonly been believed that most antibiotics display a tissue-to-plasma ratio of almost 1, several reports have indicated that target site drug levels may be substantially lower than the corresponding plasma levels (Joukhadar et al., 2001a). Suboptimal target site concentrations of antibiotics may have important clinical implications, which are one explanation for therapeutic failure in many cases (Brunner et al., 2000; Joukhadar et al., 2001b); it is also conceivable that subinhibitory concentrations in tissues may also trigger bacterial resistance (Herkner et al., 2002).

Therefore, it is necessary to use the optimal dosage of the antibiotic(s) to ensure proper exposure at the site of infection (Nicasio et al., 2009). Recently, drug delivery to the lungs by inhalation has attracted a great deal of scientific and biomedical interest. Inhalation therapy is widely employed to deliver drugs to the respiratory epithelium, mainly for the treatment of local disorders (Learoyd et al., 2008). Aerosolized antibiotic agents have been used for the treatment and prophylaxis of pulmonary infections since the 1950s mainly due to the fact that targeted drug delivery can produce high concentrations at the site of infection in the lung while minimizing the systemic toxicity (Hagerman et al., 2006). In the past decades, the topical application of aerosolized antibiotics has been used for a number of compounds and indications, e.g. gentamicin (Goldman et al., 1990; Twiss et al., 2005), tobramycin (Pai and Nahata, 2001; Pilcer et al., 2006), capreomycin (Giovagnoli et al., 2007), moxifloxacin (Ventura et al., 2008), rifampicin (Ohashi et al., 2009) and ciprofloxacin (Li et al., 2001; Arnold et al., 2007).

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Dry powder inhalers (DPI) are emerging as a preferred drug delivery method compared with metered dose inhalers and nebulizers due to the improved stability of the dry powders and the ease of use of the device (El-Gendy and Berkland, 2009). Traditional DPI formulations associate the use of micronised drug, usually 1–5 μm in diameter, with larger (50–200 μm), inert crystalline carrier materials to form ordered mixes. On inhalation, the drug particles are liberated from the carrier and penetrate the lung (Young et al., 2002). In most dry powder formulations, drug particles are usually present in low concentrations, with a drug to carrier ratio of 1:67.5 (w/w) being typical (Zeng et al., 1998). In fact, most antibiotics have a lower potency and consequently require a larger dose (e.g. oral administration of ciprofloxacin 300 mg b.i.d. and oral administration of azithromycin 500 mg q.d.). Although administration of aerosolized antibiotics by traditional DPI is likely to achieve therapeutic effects with smaller drug doses as compared with the enteral or parenteral route, traditional DPI formulations are unable to meet the requirement for a high dose of antibiotic.

In addition to dosage, there is the other issue worthy of consideration – lung deposition in patients. As we know, the respiratory pattern of patients during aerosol intake may influence the deposition of inhaled particles, because the mean flow rates of the particles in each region of the airways are governed by the breathing volume and frequency of breathing (Islam and Gladki, 2008). The amount of deposition of an inhaled drug in an individual patient's lungs is variable, depending on the overall inspiratory flow rate the patient can generate at a given time (Son and McConville, 2008). Accordingly, if the amount of lung deposition is low, the efficacy of inhalation therapy will be low or zero. Conversely, if too much lung deposition is achieved, as for some drugs with a narrow therapeutic window, this could be potentially very dangerous. For example, aminoglycoside antibiotics are concentration-dependent agents. The higher the concentration at the target site, the more potent the effect is. Above the therapeutic window, however, they may cause severe ototoxicity and nephrotoxicity during long-term therapy (Pilcer et al., 2006). To date, some advances in DPI devices have also seen the development of active devices which provide energy to assist the patient receive the correct dose (Atkins, 2005). Active DPIs overcome problems associated with dependence upon inspiratory air flow exhibited by many of the present passive devices (Islam and Gladki, 2008). However, this is generally accompanied by a higher cost.

To take these factors into account, azithromycin (AZI) was selected for pulmonary delivery. Firstly, AZI is a broad-spectrum antibiotic. It has been found to be effective against a variety of different Gram-positive and Gram-negative bacterial pathogens, and some activity against *Haemophilus influenzae* and the atypical organisms *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Legionella pneumophila*, which are some of the most common causes of pneumonia (O'Doherty and Muller, 1998; Mitchell, 2005). Secondly, AZI is of time-dependent antibiotic. It exerts an optimal bactericidal effect when drug concentrations are maintained above the minimum inhibitory concentration (MIC). Therefore, as long as there is a certain amount of drug able to reach the lung, it can exhibit an inhibitory effect because its concentration exceeds the MIC for the microorganism. This effect correlates with the retain time (t > MIC). The longer the retain time at the target site, the stronger the effect is. Finally, of all the macrolide antibiotics, AZI has the strongest post-antibiotic effect (PAE) up to 2.3–4.7 h (Kasahara et al., 2004), resulting in a relatively short treatment period. Also, as far as pneumonia is concerned, long-term treatment is not needed. These factors greatly reduce the risk of lung damage.

In this study, high azithromycin loading dry powders were prepared by cospray-drying azithromycin (AZI) with a bulking agent (mannitol), a dispersibility enhancer (L-leucine) and an anti-electrostatic agent (poloxamer 188) in different ratios. Based on the characterization of these powders, the dependence of the *in vitro* aerosolization performance on the particle components and their physical characteristics were evaluated with a view to produce an optimum formulation suitable for inhalation using a five-level three-factorial central composite experimental design. Furthermore, microdialysis studies were performed to determine the local pharmacokinetics and site-specific target efficiency of the optimum AZI DPI by comparing lung epithelial lining fluid (ELF) and blood AZI levels after intratracheal administration of AZI with those after intravenous administration.

2. Materials and methods

2.1. Materials

AZI raw material (purity: 95.5%) was purchased from Shanghai Modern Pudong Pharmaceutical Co., Ltd (Shanghai, China); mannitol and L-leucine were obtained from Bodi Chemicals (Tianjin, China); poloxamer 188 (Lutroly F68) was kindly donated by BASF (Shanghai, China). Other reagents were of analytical or chromatographic grade. Double distilled water was used throughout the study.

2.2. Preparation of AZI powders for inhalation

A quantity of AZI raw material was weighed and added to a suitable volume of distilled water, and hydrochloride acid was then added dropwise with continuous stirring until a clear solution was obtained. The accurately weighed carriers were dissolved in distilled water and then mixed with the drug solution. The pH of the obtained solution was adjusted with saturated sodium hydroxide solution to 7.0, then diluted to volume and passed through a 0.22-μm cellulose acetate filter. The final solution was spray-dried using an SD-1000 spray dryer (EYELA, Japan) under the following operating conditions: inlet temperature, 120 °C; atomization pressure, 180 kPa; feed rate, 6 mL/min; aspirator setting, 0.6 m^3/min. Once the aqueous solution was used up, the outlet temperature was maintained at ~80 °C for approx. 15 min by regulating the inlet temperature so as to provide a secondary drying period. The resulting dry powders were stored in a vacuum desiccator over silica gel until required for use.

2.3. Powder characterization

2.3.1. Spray-drying yield and drug content

The yields of spray-dried powders were quantified as a percentage mass of the anticipated total powder yields. The AZI content of each prepared powder was measured in triplicate by HPLC (Zhang et al., 2009), and expressed as a percentage of the total dry powder mass.

2.3.2. Scanning electron microscopy

Selected samples of dry powder formulations were sputter layered with gold. Representative scanning electron micrographs of the powders were taken using a ZEISS SUPRA 35 scanning electron microscope.

2.3.3. Powder flowability

The most frequently employed method for characterizing the powder flowability is the angle of repose. Powders were poured through a funnel to form a cone-shaped pile which had an angle, α, to the horizontal. The value of α was calculated by measuring the height and radius of the pile. A large angle of repose is

indicative of poor flow properties while a small angle of repose indicates a free-flowing powder. The angle of repose of powders was measured using a Powder Characteristics Tester PT-R (Hosokawa Micron Corp., Japan).

2.3.4. Particle size and powder tapped density

The particle size was measured with a laser diffractometer (LS 230, Backman Coulter, USA) in dry powder form after dispersing with compressed air. Approximately 100 mg of each powder was used to achieve the required obscuration of 5%, and each sample was measured in triplicate. The data obtained were expressed as the volume weighted mean particle size. The tapped powder density (ρ) was determined by tap density measurements using a self made tapped density analyzer, which involved 1000 strokes to allow the density to reach a plateau (Bosquillon et al., 2004b). Theoretical estimates of the particle primary aerodynamic diameter (d_a) can be derived from the particle sizing and tapped density data, according to Eq. (1) (Rabbani and Seville, 2005):

$$d_a = d\left(\frac{\rho}{\rho 0}\right)^{1/2} \quad (1)$$

where d is the geometric mean diameter obtained from particle size analysis, ρ is the tapped density and ρ_0 is the reference density of 1 g/cm^3.

2.3.5. In vitro powder deposition

The pulmonary deposition of the dry powders was investigated *in vitro* using a Twin Stage Impinger (TSI, custom made). Water was introduced to the upper (7 mL) and lower (30 mL) stages of the TSI. Powders (30 mg) were loaded into size 3 HPMC capsules and placed in an Aerolizery inhaler (Schering, Co.). The capsules were pierced and the liberated powder was drawn through the TSI at a flow rate of 60 L/min for 10 s. In all cases ten capsules were discharged into the apparatus per determination and each experiment was repeated in triplicate. After each determination, the lower stage was rinsed with water and made up to a final volume of 50 mL. The amount of AZI in the washings was determined by HPLC. The respirable dose (RD) was defined as the mass of drug recovered from the lower stage of the TSI (effective cut-off diameter 6.4 μm). The respirable fraction (RF), defined as the ratio of the RD to the total loaded dose, was expressed as a percentage and corrected for the actual AZI content of each powder (Rabbani and Seville, 2005).

2.4. Experimental design

A five-level three-factorial central composite rotatable design (CCRD) (Design Expert, Version 7.1.3, Stat-Ease Inc., Minneapolis, MN) was used for conducting the experiments. CCRD requires many fewer tests than the full factorial design and has been shown to be sufficient to describe the majority of steady-state process responses. The following polynomial equation was fitted to the data:

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{13}X_1X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 \quad (1)$$

where Y represents the response associated with each factor level combination, b_0 is an intercept and b_1–b_{33} is the regression coefficients of the factors (Chopra et al., 2007). The levels of each variable were designated as −1.68, −1, 0, +1 and +1.68, respectively, and the corresponding actual values for each variable are listed in Table 1.

Table 1
Variables in the central composite experimental design.

Factor	Level				
	−1.68	−1	0	+1	+1.68
X_1: mannitol (%, w/v)	0.38	1.2	2.4	3.6	4.42
X_2: L-leucine (%, w/v)	0.19	0.6	1.2	1.8	2.21
X_3: F68 (10^{-3}%, w/v)	6.36	20	40	60	73.64

Response	Constraints
Y_1: angle of repose (°)	Minimize
Y_2: aerodynamic diameter (μm)	Minimize
Y_3: RF (%)	Maximize
Y_4: yield (%)	Maximize

2.5. In vivo studies

2.5.1. Animal model

All animal experiments were performed in strict accordance with the protocol approved by the Institutional Animal Care and Use Committee of Shenyang Pharmaceutical University. Male Sprague-Dawley rats weighing between 220 and 250 g were anesthetized with an intraperitoneal injection of 0.2 g/mL urethane (1.4 g/kg body weight). Each anesthetized rat was placed on its back with its head towards the investigator, and a 6.5 cm microdialysis probe was then advanced through the tracheostomy at an angle of about 10° towards the deep lung as described by Eisenberg et al. (1993). Subsequently, the blood microdialysis probe was implanted within the jugular vein toward the right atrium and, at the same time, two probes were perfused with modified Ringer's solution (147 mM NaCl, 2.2 mM $CaCl_2$, 4.0 mM KCl, and pH 7.2) (de Lange et al., 2000) and ACD (3.5 mM citric acid, 7.5 mM sodium citrate and 13.6 mM dextrose) (Wu et al., 2004) separately, at a flow rate of 3 μl/min, using the S200 Microdialysis syringe pump (KD Scientific Company, Houston, TX) for both delivery of the perfusion solution and sample collection.

2.5.2. Drug administration and collection of biological samples

Twelve rats were divided into two groups. Drug administration in each group was performed following the successful implantation of the microdialysis probe and equilibrium for 1.0 h with perfusion of Ringer's and ACD solutions. One group was given AZI DPI (23 mg/kg) via intratracheal administration. A 2.0-cm length of PE-240 polyethylene tubing was inserted to a depth of 0.6 cm through the tracheostomy. AZI dry powder was administrated as previously reported (Todo et al., 2003) using the apparatus. In brief, a certain amount of dry powder was put in a disposable tip and dispersed in the rat trachea by releasing air compressed (4.0 mL) in a syringe by quickly opening the three-way stopcock connecting the tip and the syringe. The amount of dry powder administered was calculated by subtracting the tip weight after administration from that before administration. Another group was given 45 mg/kg AZI solution by intravenous injection. After administration, lung and blood dialysis samples were collected at intervals of 20 min for 6.0 h, and the samples were kept frozen at 20 °C and analyzed directly by UPLC-MS/MS within 48 h according to the method of Chen (Chen et al., 2007).

3. Results and discussion

3.1. Preparation of the dry powders

Dry powders were usually prepared by spray-drying using GRAS (generally recognized as safe) excipients. So far, lactose is the only excipient that is FDA-approved for inhalation largely because it is a safe pharmaceutical excipient, readily available,

physico-chemically stable and compatible with the majority of small molecular weight drugs. There are many other pharmaceutical excipients, particularly some sugars and polyols, which also meet the criteria required for incorporation as a suitable carrier in dry powder aerosols. In this study, mannitol was chosen because not only it has been used widely in the pharmaceutical industry, but also it is the active ingredient of Bronchitol (320 mg twice daily), which is the key Pharmaxis product in development. Mannitol hydrates the lungs, helps restore normal lung clearance, and allows patients to clear mucus more effectively. A Phase 3 clinical study has shown that mannitol is safe, effective, and well tolerated when treating patients with both cystic fibrosis and bronchiectasis. A second Phase 3 clinical trial is in progress in sites around the world and this will study the effects of mannitol on reducing the infectious episodes, antibiotic use and length of hospitalization for patients with bronchiectasis. Furthermore, compared with lactose, mannitol has a better effect on powder hygroscopicity as reported in our earlier paper (Zhao et al., 2008).

In order to deliver non-viral gene vectors via dry powder inhalers, Seville et al. added various amino acids to the spray-drying solution before powder processing. This was shown to significantly improve the *in vitro* deposition of these bioactive macromolecules with significant quantities of powder deposition in regions of the lower respiratory tract. They also proved that the amino acids performed well in enhancing the *in vitro* deposition of low molecular weight drugs (Li et al., 2003, 2005; Seville et al., 2007). In our preliminary experiments, acidic amino acids (aspartic acid), basic amino acids (lysine and arginine), hydrophobic amino acids (alanine, valine, phenyalanine, leucine and isoleucine), and hydrophilic amino acids (glycine and threonine) were investigated as potential "dispersibility enhancers" in spray-dried powders containing mannitol as a primary excipient or auxiliary active ingredient and AZI as a low molecular weight model drug. The effect of amino acids on the *in vitro* deposition of each formulation was arranged in descending order: leucine, isoleucine, glycine, lysine, valine, alanine, aspartic acid, threonine, arginine and phenyalanine. Specifically, leucine showed the strongest effect mainly due to its surfactant-like properties (Gliniski et al., 2000; Seville et al., 2007). Apart from leucine, another surfactant, poloxamer 188 (F68) was used to control the surface properties of micronized particles and, thus, prevent the particles from agglomerating and becoming highly charged.

AZI is the first clinically developed antibiotic in a new subclass of macrolides, the azalides, characterized by the expansion of the 14-membered aglycone ring of erythromycin with an endocyclic ionizable nitrogen. AZI is derived from erythromycin, which differs from erythromycin by the insertion of a methyl-substituted nitrogen in the lactone ring. This modification produces an enhanced spectrum and potency against bacteria compared with other macrolides and superior stability in an acidic environment (Zhang et al., 2009). AZI is poorly soluble in water at 25 °C.

Table 2
Observed responses for the central composite rotatable design.

Run	X_1	X_2	X_3	Y_1	Y_2	Y_3	Y_4
1	3.60	0.60	60.00	53.75	14.36	7.88	52.14
2	4.42	1.20	40.00	43.39	6.68	21.37	48.03
3	2.40	1.20	6.36	42.27	7.39	31.87	56.76
4	1.20	1.80	20.00	57.09	6.73	43.79	62.94
5	3.60	1.80	60.00	47.49	5.52	28.56	62.46
6	0.38	1.20	40.00	45.77	5.96	42.72	57.93
7	1.20	0.60	60.00	48.88	8.89	23.91	49.75
8	2.40	0.19	40.00	56.78	11.94	10.70	35.38
9	2.40	1.20	73.64	40.82	6.82	27.67	59.13
10	3.60	0.60	20.00	51.84	8.43	13.29	44.62
11	3.60	1.80	20.00	48.65	6.32	32.09	55.99
12	2.40	1.20	40.00	38.66	7.00	35.25	66.49
13	2.40	1.20	40.00	38.02	7.71	32.61	63.21
14	1.20	0.60	20.00	47.49	5.75	31.90	58.24
15	1.20	1.80	60.00	53.75	6.15	40.68	62.29
16	2.40	2.21	40.00	58.57	7.10	38.05	59.57
17	2.40	1.20	40.00	39.29	6.98	37.65	65.08

However, as calculated using Advanced Chemistry Development (ACD/Labs) Software V8.14 for Solaris, the mass solubility of AZI reaches 1000 g/L over the range of pH 1–6 at 25 °C and is 100 g/L at pH 7, 25 °C. Accordingly, different kinds of acids were added dropwise to obtain a clear solution during the preparation process. The pH of the solution was then adjusted with saturated sodium hydroxide solution to 7.0, which was reported to be suitable for the lung fluid environment (Schanker and Less, 1977) and no drug precipitation was seen. Both monoacids (hydrochloride acid and glacial acetic acid) and polybasic acids (phosphoric acid, sulphuric acid, and citric acid) were investigated in our study. As a result, the powder with hydrochloride acid as a pH modifier was found to disperse better and provide a higher *in vitro* deposition. This might be related to the sodium chloride, which was generated by the neutralization reaction between hydrochloride acid and sodium hydroxide. Chan et al. (1997) have reported that as the NaCl content increases, the morphology of the powder changes from spheres with a smooth surface texture to spheres with a surface having many apparently salt crystals. It is known that amorphous powders generally have a higher surface energy giving rise to direct attractive forces between particles and to the adsorption of water leading to capillary inter-particle forces compared with crystalline powders.

3.2. Central composite rotatable design (CCRD) and analysis

In the present study, seventeen experiments were conducted in one block, composed of eight factorial points, six axial points and three central points. Three variables were considered: mannitol (X_1), L-leucine (X_2) and F68 (X_3). All the formulations were prepared from solutions with the same AZI concentration (6%) following

Table 3
The analysis of variance for responses Y_1, Y_2, Y_3 and Y_4.

Source	Y_1		Y_2		Y_3		Y_4	
	F-value	*P*-value, prob > *F*	*F*-value	*P*-value, prob > *F*	*F*-value	*P*-value, prob > *F*	*F*-value	*P*-value, prob > *F*
Model	23.45	0.0002	8.17	0.0057	61.32	<0.0001	8.99	0.0043
X_1	1.97	0.2029	4.81	0.0643	192.92	<0.0001	7.20	0.0314
X_2	1.42	0.2728	30.42	0.0009	282.15	<0.0001	37.97	0.0005
X_3	0.30	0.6031	3.17	0.1184	15.91	0.0053	0.47	0.5160
X_1X_2	21.46	0.0024	10.08	0.0156	4.33	0.0760	0.20	0.6663
X_1X_3	0.27	0.6171	0.79	0.4040	0.17	0.6894	5.47	0.0519
X_2X_3	2.28	0.1745	13.05	0.0086	1.69	0.2350	0.47	0.5144
X_1^2	24.24	0.0017	0.98	0.3550	5.04	0.0596	10.28	0.0149
X_2^2	180.60	<0.0001	7.43	0.0295	51.87	0.0002	25.76	0.0014
X_3^2	8.69	0.0215	0.006	0.9404	13.83	0.0075	2.31	0.1723
Lack of fit	11.16	0.0843	8.07	0.1139	0.35	0.8540	5.92	0.1508

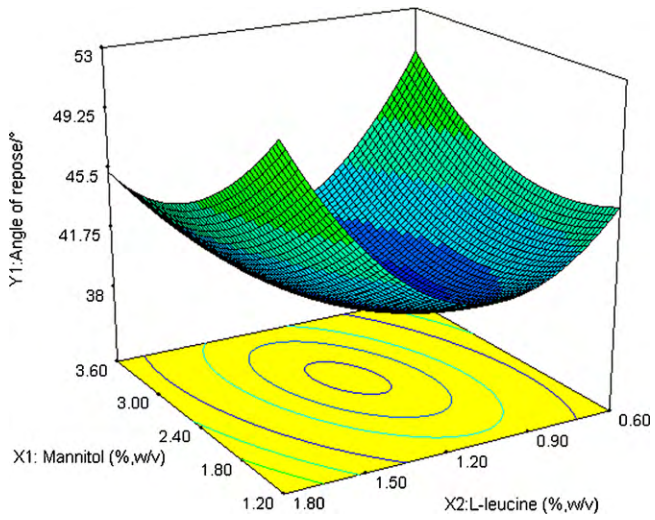

Fig. 1. Response surface plot showing effects of X_1 and X_2 on response Y_1.

the same procedure and spray-drying conditions. The considered responses were the angle of repose (Y_1), aerodynamic diameter (Y_2), RF (Y_3) and yield (Y_4) of the dry powder. Response data for all experimental runs of CCRD are presented in Table 2 and fitted to first-order, two-factor interaction and quadratic models. For the estimation of the significance of the model and term, analysis of variance (ANOVA) was carried out (Table 3). The probability P-value of each model was relatively low ($P < 0.05$), indicating the significance of the model. The resulting equations and the corresponding R^2-values are presented below:

$$Y_1 = 38.49 - 0.69X_1 + 0.59X_2 - 0.27X_3 - 2.99X_1X_2 + 0.34X_1X_3 - 0.98X_2X_3 + 2.68X_1^2 + 7.31X_2^2 + 1.60X_3^2, \quad R^2 = 0.9679$$

$$Y_2 = 7.23 + 0.61X_1 - 1.53X_2 + 0.49X_3 - 1.15X_1X_2 + 0.32X_1X_3 - 1.31X_2X_3 - 0.30X_1^2 + 0.83X_2^2 - 0.024X_3^2, \quad R^2 = 0.9131$$

$$Y_3 = 35.20 - 6.91X_1 + 8.36X_2 - 1.98X_3 + 1.35X_1X_2 + 0.27X_1X_3 + 0.84X_2X_3 - 1.23X_1^2 - 3.94X_2^2 - 2.04X_3^2, \quad R^2 = 0.9875$$

$$Y_4 = 64.67 - 2.54X_1 + 5.83X_2 + 0.65X_3 + 0.56X_1X_2 + 2.89X_1X_3 + 0.85X_2X_3 - 3.34X_1^2 - 5.28X_2^2 - 1.58X_3^2, \quad R^2 = 0.9203$$

The R-squared ($R^2 > 0.9$) indicates a high correlation between the experimentally observed and predicted values. According to the equations, three dimensional response surfaces and two-dimensional contours were plotted and presented in Figs. 1–4. These types of plots show the effects of two factors on the response at any time. In all the presented pictures, the third factor was kept at level zero.

As shown in Fig. 1, at a low level of L-leucine, the angle of repose decreased slightly, and then increased with the increasing the amount of mannitol. Conversely, at a high level of L-leucine, the angle of repose decreased with the mannitol content. Previous research has shown that at 10–20% mannitol, the spray-dried powders remained amorphous, whereas the 30% mannitol sample crystallized at both 5 and 30 °C following a 1-year storage period. It should be noted that at a higher level of mannitol, i.e. 40%, prior to storage, the powder exhibited an obvious crystalline

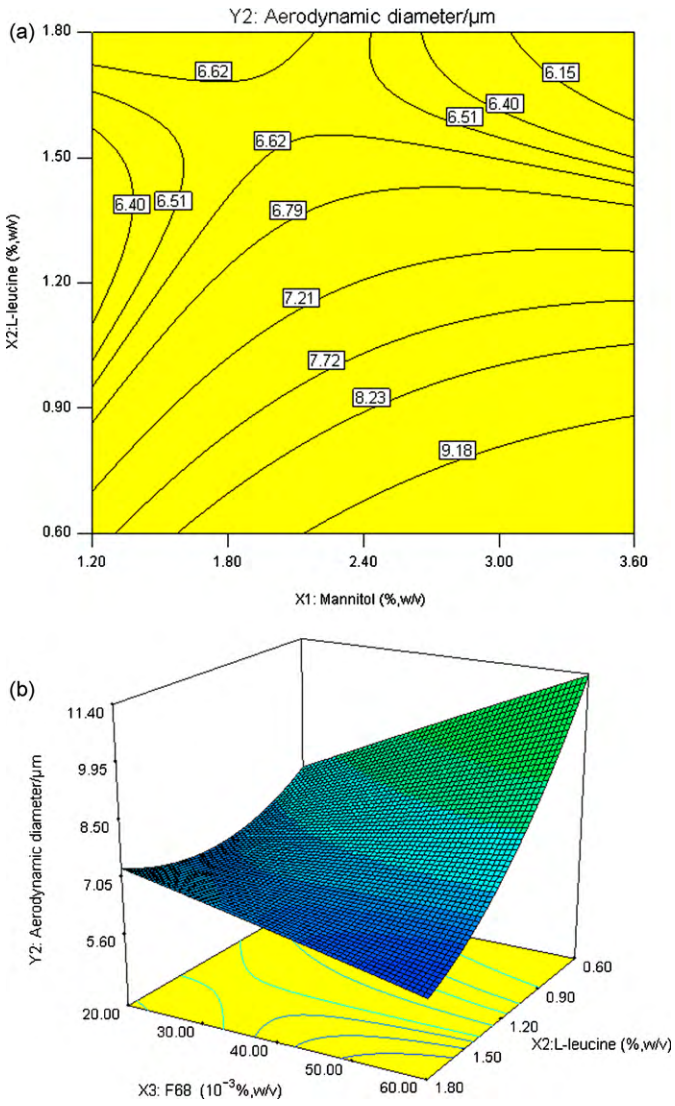

Fig. 2. The contour plot (a) and response surface plot (b) showing effects of X_1, X_2 and X_3 on response Y_2.

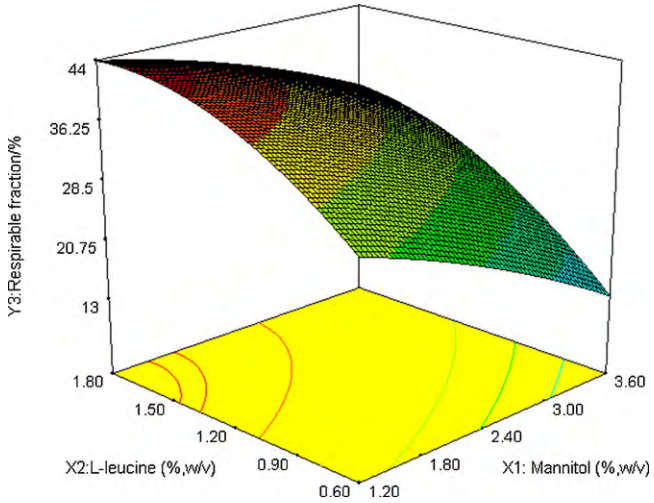

Fig. 3. Response surface plot showing effects of X_1 and X_2 on response Y_3.

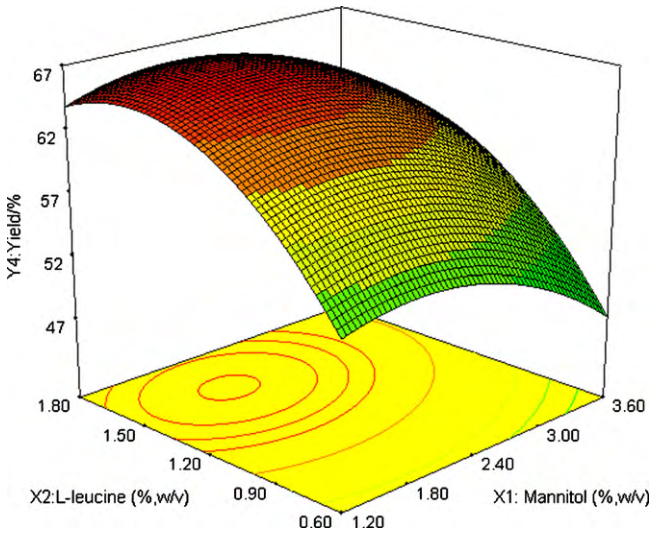

Fig. 4. Response surface plot showing effects of X_1 and X_2 on response Y_4.

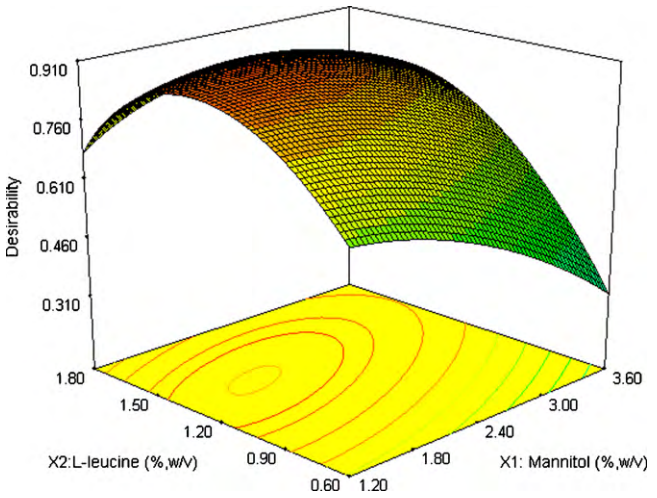

Fig. 5. The relation between overall desirability and variables X_1 and X_2.

character (Costantino et al., 1998). It is supposed that at a low fraction of L-leucine, mannitol crystals on the surface of the powders appear and increase on increasing the amount of mannitol. A small amount of crystals reduce the surface energy, which is favorable for the flowability. However, a large number of crystals are likely to increase the particle size and inter-particle growth, which adversely affect the aerodynamic diameter and flowability (see Fig. 2a). Alternately, on increasing the fraction of L-leucine, its hydrophobic and surfactant-like properties may play a major role. This allows L-leucine to migrate to the droplet surface during the rapid drying phase of the spray-drying and, hence, influence the surface characteristics of the resulting particles (Gliniski et al., 2000; Seville et al., 2007). Furthermore, L-leucine is able to act as an anti-adherent in the case of dry powders. It is assumed that L-leucine interferes with the weak bonding forces, such as Van der Waal's and Coulomb forces, between the small particles which helps to keep the particles separated and these may be thought of as weak links or "chain breakers" between the particles (Chougule et al., 2007). These particular properties of L-leucine give the powders a better flowability and produce smaller particles, which also has a positive effect on the aerodynamic diameter (see Fig. 2a). Nevertheless, higher concentrations of leucine might lead to highly charged particles, thereby adversely affecting the powder flow properties. The formulation would be diluted with the increase of mannitol. And the relative ratio of leucine (or relative concentration of leucine) would also be reduced. Naturally, the increase of mannitol prevents excessive surface charge and improves the flowability to some extent. Similarly, in Fig. 2b, the function of F68 is to control the surface properties of micronized particles and, thus, prevents the particles from agglomerating and becoming highly charged. Steckel and Brandes have reported that F68 plays a special role in determining the spray-dried particle morphology (Steckel and Brandes, 2004). They found that increasing the amount of F68 affected the flowability and dispersibility of the powders as a higher number of fine particle fractions was obtained. However, even higher concentrations of F68 still lead to highly charged particles. Compared with L-leucine, a small amout of F68 will be more efficient. This is mainly because L-leucine is a very weak surfactant (Gliniski et al., 2000).

The most important response is the *in vitro* deposition (expressed as RF). More often than not, an *in vitro* deposition test is used to simulate the *in vivo* inhalation process of DPI. As displayed in Fig. 3, L-leucine has a positive effect on RF, whereas mannitol has a negative effect. Thus, combining more L-leucine with less mannitol resulted in a relative higher concentration of L-leucine in the total dry powder mass and then a higher RF. The contribution of L-leucine to the improved aerosolization could be due to its surfactant-like and anti-adherent properties. When agglomerates of particles are formed, the addition of L-leucine reduces the stability of those agglomerates so that they are more likely to break up in the turbulent air stream created on inhalation to form small individual particles which are likely to reach the lower lung (Chougule et al., 2007).

In terms of the yield, the dry powders showed great variations (Fig. 4). It has previously been suggested that low spray-drying yields are indicative of cohesive powders that demonstrate poor aerosolisation properties, and the addition of formulation excipients may change the physicochemical characteristics of the resultant spray-dried powders, resulting in an improved spray-drying yield and potentially enhancing the aerosolisation properties (Li et al., 2005). Alternatively, low spray-drying yields may be a reflection of highly adhesive particles resulting in adhesion of the powders to the walls of the spray-drier (Seville et al., 2007). In this study, the addition of L-leucine to the spray-drying solution led to an initial increase in powder yield. However, a further increase in the amount of L-leucine resulted in a decrease in spray-drying yield. A similar pattern could be observed for mannitol. As a result, in order to obtain a formulation with a maximized spray-drying yield, the optimum fraction of L-leucine and mannitol content was required to coordinate the relationship between L-leucine and mannitol, as described before.

3.3. Optimization and characteristics

The optimum formulation was selected based on the criteria for attaining the minimum angle of repose (Y_1) and aerodynamic diameter (Y_2), while maximizing RF (Y_3) and yield (Y_4). An overall desirability function dependent on all the investigated formulation variables was used to predict the ranges of variables where the optimum formulation may occur (Li et al., 2008). The desirable ranges are from 0 to 1 (least to most desirable, respectively). The optimization procedure was conducted automatically by the Design Expert 7.1.3. The relationship between the overall desirability and variables X_1, X_2 is described by Fig. 5. To check the validity of the calculated optimal factors and predicated responses, a new batch with the optimized formula was prepared and evaluated in triplicate. Table 4 illustrates the predicated and observed responses for the optimum formulation. The overall desirability (OD) is 0.908,

Table 4
The observed and predicted response values for the optimized formulation.

Factor	Optimized level		
X_1: mannitol (%, w/v)	1.82		
X_2: L-leucine (%, w/v)	1.35		
X_3: F68 (10^{-3}%, w/v)	38		
Overall desirability	0.908		
Response	Expected	Observed	Residual
Y_1: angle of repose (°)	40.5	39.3	−1.2
Y_2: aerodynamic diameter (μm)	6.67	6.39	−0.28
Y_3: RF (%)	40.1	40.5	0.4
Y_4: yield (%)	66.2	66.0	−0.2

which is close to 1. Furthermore, there is less residual between the predicated and observed responses. This suggests that the optimum formulation is desirable.

Many researchers have found that large particles (aerodynamic diameter, $d_a > 5$ μm) are deposited by inertial impaction in the mouth and upper airways, while smaller particles ($d_a = 1–5$ μm) are deposited deeper in the lungs by inertial impaction and sedimentation, whereas very small particles ($d_a < 1$ μm) are driven by diffusion, mostly remain suspended and are then exhaled. However, the d_a of the optimum formulation is beyond 5 μm, which adversely deposits in the deeper regions of the lungs. In order to obtain a suitable particle size, the spray-drying conditions were further optimized. In our study, it was noted that reducing the feedstock concentration resulted in smaller particles, which agreed with previous reports (Corrigan et al., 2004, 2006; Mosen et al., 2004). Finally, the feedstock solution containing 6.0 g AZI, 1.82 g mannitol, 1.35 g L-leucine and 38 mg F68 was diluted to 400 mL and then spray-dried under the following conditions: inlet temperature, 120 °C; airflow rate, 0.70 m^3/min; atomizing pressure, 190 kpa; feedstock rate, 4 mL/min. The optimized powder, which had a better flowability (repose of angle, 36°), an aerodynamic diameter of 3.82 μm, an *in vitro* deposition of 51% and a high yield of 64.9%, was obtained under above spray-drying conditions. The content of the powder was high (59.2%), which met the requirement for high drug loading.

The SEM image is shown in Fig. 6a. The optimized powder exhibited hollow spherical particles with an extremely wrinkled surface. This can be explained by the surfactant-like properties of leucine and the hydrophobic nature, which made it likely to accumulate at an air/liquid interface. Leucine molecules probably form a relatively hydrophobic crust, through which the solvent located in the interior of the droplets cannot easily pass, resulting in a build-up of pressure within the droplets and their subsequent collapse (Seville et al., 2007). Some completely collapsed balloons were evidence to support this. The advantage of wrinkled, hollow particles is that they have a reduced tapped density and there is less contact between particles, thereby resulting in a better *in vitro* deposition. In addition, as shown in Fig. 6b, a few salt crystals were present on the surface of the powders. This may be sodium chloride generated by hydrochloride acid and sodium hydroxide, which supported our above hypothesis.

Finally, an important aspect for *in vivo* applications concerns the ability of particles to readily flow out of the apparatus for intratracheal administration and generate a fine aerosol so that a proper dose can reach the deeper lung regions of rats. The image of 10 mg optimal powder delivery, recorded by means of a digital videocamera, showed that the particles readily flowed out of the apparatus generating a fine aerosol (Fig. 7). Also, there appeared to be no aggregation of the powder exiting the apparatus. It is suitable for inhalation administration and applied in the *in vivo* study.

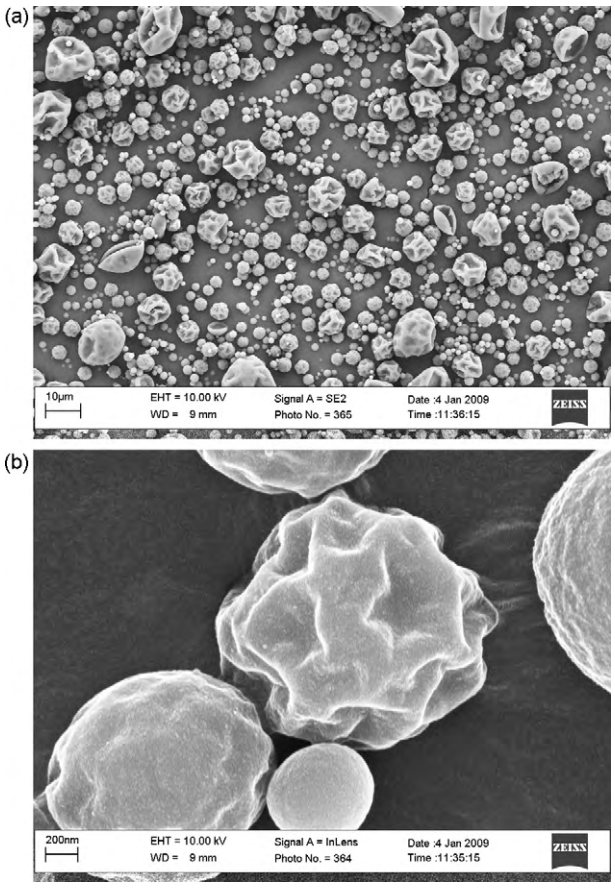

Fig. 6. Scanning electron micrographs of optimal powders (a) ×2k and (b) ×100k.

3.4. In vivo study

To estimate the true concentrations of AZI in the ELF and blood, the relationships between AZI concentrations in the dialysis samples and the ELF (or blood) surrounding the microdialysis membrane (*in vivo* recovery) had to be established. Microdialysis probe *in vivo* recovery was calibrated by measurements of *in vitro* recovery ($R_{in\ vitro}$), *in vitro* delivery ($D_{in\ vitro}$) and *in vivo* delivery ($D_{in\ vivo}$). $R_{in\ vitro}$ and $D_{in\ vitro}$ were determined in *in vitro* simulated experiment, while a retrodialysis method was performed to determine the *in vivo* delivery of AZI in three blank rats. Therefore, *In vivo* recovery was calculated according to $(R/D)_{in\ vitro} = (R/D)_{in\ vivo}$.

Fig. 7. High-speed photograh of optimal powder exit from the device loaded with 10 mg powder.

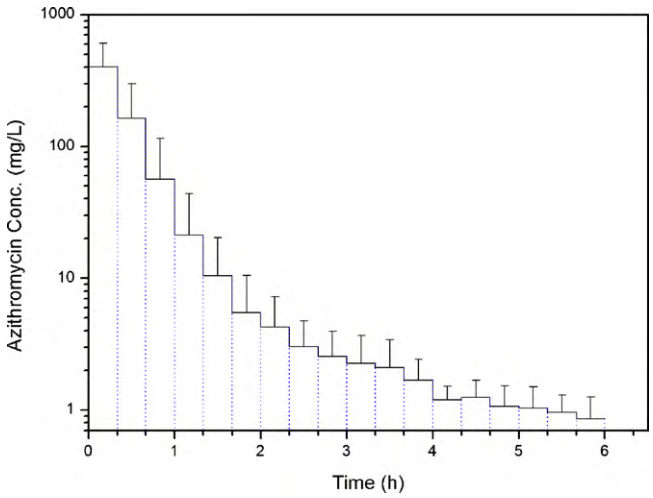

Fig. 8. Mean concentration–time profile of AZI in rat ELF after intratracheal administration at the dose of 23 mg/kg DPI ($n = 6$).

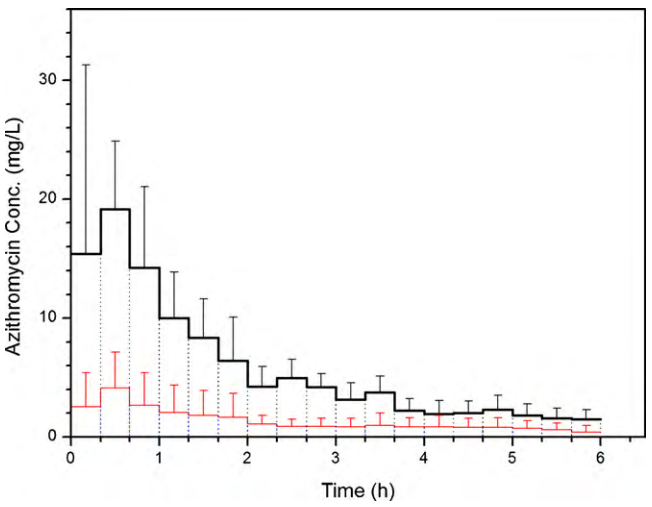

Fig. 9. Mean concentration–time profile of AZI in rat blood after intratracheal (thin line style) and intravenous (thick line style) administration at the dose of 23 mg/kg DPI and 45 mg/kg AZI solution, respectively ($n = 6$).

The concentrations of AZI in the ELF and blood were adjusted by the individual *in vivo* recoveries. Fig. 8 shows the concentration–time profile of AZI in rat ELF after intratracheal administration of AZI DPI. AZI achieved a high local concentration in ELF initially, subsequently fell rapidly over 2 h, and was then eliminated slowly. According to the method of Rennard (Rennard et al., 1986), urea was selected as the marker of the ELF to determine the actual volume of ELF in our study. It was calculated that the actual volume of ELF was 232.7 ± 59.2 μL ($n = 14$, rats: 220–270 g). Thus, some particles dissolved in a small volume of ELF, resulting in a high local AZI concentration. The other particles (especially with a geometric size 1–5 μm) may be taken up by the resident macrophages as early as 1 h post-delivery (Bosquillon et al., 2004a). In the meantime, AZI dissolved in the ELF could rapidly penetrate and accumulate in alveolar macrophages. The uptake of AZI into macrophages is attributed to its dibasic amphophilic nature, a feature common to lysosomotrophic agents. Within hours, the intracellular concentrations of AZI can exceed the extracellular concentrations 100-fold, and then a further gradual increase occurs (Hoepelman and Schneider, 1995). This results in the rapid decline in the AZI concentration in the ELF within the first two hours and then a slow elimination. Because of macrophage phagocytosis, only a few AZI molecules are able to reach the bloodstream through the pulmonary capillaries. It results in a rather low blood concentration of AZI after intratracheal administration compared with that after intravenous administration, as is shown in Fig. 9.

It is well known that the pharmacokinetics of AZI is characterized by very low blood concentrations and wide tissue distribution. The tissue half-life is estimated to be greater than 2 days. It is assumed that AZI achieves high concentrations in phagocytic cells such as polymorphonuclear leukocytes (PMNs) and monocytes. AZI uptake by both PMNs and monocytes showed an intracellular to extracellular concentration ratio (I/E) of ~200 (Hall et al., 2002). PMNs account for 50–70% of white blood cells, while monocytes account for only 7%. From the peripheral blood, monocytes migrate to extravascular tissue where they differentiate into macrophages. Macrophages colonize the liver (Kupffer cells), lungs (alveolar macrophages), spleen, lymph nodes, thymus, gut, marrow, brain, connective tissue and serous cavities (Ahsan et al., 2002). PMNs remain in the peripheral blood for 6–12 h, and then enter the extravascular tissue. The aging PMNs will be phagocytozed and decomposed by macrophages. Also, the release of AZI from phagocytes is extremely slow in contrast with that observed for erythromycin (Hoepelman and Schneider, 1995). AZI is a weakly basic (dibasic) antibiotic that can be protonated and trapped within acidic lysosomes, accounting for its prolonged cellular retention (Hand and Hand, 2001). This was further confirmed by the low concentration of AZI in the ELF after intravenous administration (Fig. 10). All these factors contribute to the unique pharmacokinetics of AZI.

Although microdialysis is a continuous sampling technique, the dialysate is most often analyzed as a series of discrete samples, collected over a finite period of time. Therefore, conceptually, a histogram is a more accurate graphic presentation of the microdialysis data than a point to point curve. Each of the rectangles forming the histogram represents a single sample with its collection time as the rectangle base and the average ELF drug concentration during the sampling interval as the rectangle height. The AUC over the total collection period is the sums of the areas of the constituent rectangles (drug concentration × sampling interval). It is more accurate and independent of the sampling frequency (Eisenberg et al., 1993). The bioavailability was calculated by dividing the mean blood AUC after intratracheal dosing by the mean value after i.v. administration. The apparent ELF availability was defined as the ratio of $AUC_{ELF,i.t.}/AUC_{ELF,i.v.}$. The degree of AZI targeting to ELF after intratracheal administration can be evaluated by the drug targeting

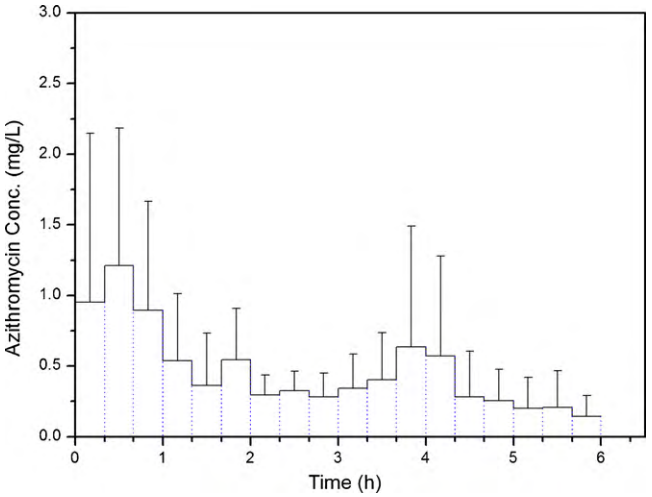

Fig. 10. Mean concentration–time profile of AZI in rat ELF after intravenous administration at the dose of 45 mg/kg AZI solution ($n = 6$).

Table 5
AUC values of AZI following intratracheal (i.t.) and intravenous (i.v.) administration ($n = 6$).

	AUC_{blood} (mg h/L)	AUC_{ELF} (mg h/L)	$\frac{AUC_{ELF}}{AUC_{blood}}$
Intratracheal (23 mg/kg)	7.95 ± 6.03	226.31 ± 121.56	42.3 ± 30.9
Intravenous (45 mg/kg)	35.72 ± 11.92	2.74 ± 1.18	0.087 ± 0.059
$\frac{AUC_{i.t.} \times dose_{i.v.}}{AUC_{i.v.} \times dose_{i.t.}}$	43.5%[a]	161.6[b]	DTI[c] = 486.2

[a] Bioavailability.
[b] Apparent ELF availability.
[c] Drug targeting index.

index (DTI), which can be described as the ratio of the value of AUC_{ELF}/AUC_{blood} following intratracheal administration compared with that following intravenous injection. The higher the DTI, the greater the degree of AZI targeting to ELF can be expected after intratracheal administration. As shown in Table 5, the bioavailability of AZI obtained following intratracheal administration was 43.5%; the apparent ELF availability was 161.6. The AUC_{ELF}/AUC_{blood} ratio (42.3 ± 30.9) after intratracheal administration differed significantly from the ratio (0.087 ± 0.059) observed after intravenous administration ($P < 0.05$). As for the DTI, the value achieved was 486.2, which is far greater than 1. The results of this study show that AZI DPI can be effectively and efficiently delivered to a specific target site and achieve a high local concentration. Like most infections, the majority of bacterial lung infections are localized to the interstitial spaces (Blumer, 2005). The FDA and the European Agency for the Evaluation of Medicinal Products (EMEA) have recognized that blood concentrations of antimicrobial agents are not of great relevance (Zeitlinger et al., 2005). The concentration of AZI in the ELF is generally regarded as a more important factor than that in blood in terms of achieving an effective clinical treatment. Thus, AZI DPI results in effective drug concentrations at the target site with minimal systemic bioavailability and side effects. This approach is able to achieve therapeutic effects with smaller drug doses compared with the enteral or parenteral route.

4. Conclusion

This paper describes a new concept for the development of DPI containing a time-dependent antibiotic, azithromycin, which has received little attention to date. The optimized powder was obtained in this study showed better flowability, a suitable aerodynamic diameter, high yield and high drug loading as well as a desirable respirable fraction. It was suitable for inhalation administration and being used in an *in vivo* study in rats. This *in vivo* study revealed that the administration of AZI offered an attractive alternative, delivering high concentrations of antibiotic directly to the target site while minimizing the systemic bioavailability and side effects. Taken together, AZI pulmonary delivery DPI is likely to be of value in future clinical applications.

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References

Ahsan, F., Rivas, I.P., Khan, M.A., Torres Suarez, A.I., 2002. Targeting to macrophages: role of physicochemical properties of particulate carriers – liposomes and microspheres – on the phagocytosis by macrophages. J. Control Release 79, 29–40.
Arnold, M.M., Gorman, E.M., Schieber, L.J., Munson, E.J., Berkland, C., 2007. NanoCipro encapsulation in monodisperse large porous PLGA microparticles. J. Control. Release 121, 100–109.
Atkins, P.J., 2005. Dry powder inhalers: an overview. Respir. Care 50, 1304–1312 (discussion 1312).
Blumer, J.L., 2005. Evolution of a new drug formulation: the rationale for high-dose, short-course therapy with azithromycin. Int. J. Antimicrob. Agents 26, 143–147.
Bosquillon, C., Preat, V., Vanbever, R., 2004a. Pulmonary delivery of growth hormone using dry powders and visualization of its local fate in rats. J. Control. Release 96, 233–244.
Bosquillon, C., Rouxhet, P.G., Ahimou, F., Simon, D., Culot, C., Preat, V., Vanbever, R., 2004b. Aerosolization properties, surface composition and physical state of spray-dried protein powders. J. Control. Release 99, 357–367.
Brunner, M., Pernerstorfer, T., Mayer, B.X., Eichler, H.G., Muller, M., 2000. Surgery and intensive care procedures affect the target site distribution of piperacillin. Crit. Care Med. 28, 1754–1759.
Chan, H.K., Clark, A., Gonda, I., Mumenthaler, M., Hsu, C., 1997. Spray dried powders and powder blends of recombinant human deoxyribonuclease (rhDNase) for aerosol delivery. Pharm. Res. 14, 431–437.
Chen, L., Qin, F., Ma, Y., Li, F., 2007. Quantitative determination of azithromycin in human plasma by ultra performance liquid chromatography–electrospray ionization mass spectrometry and its application in a pharmacokinetic study. J. Chromatogr. B 855, 255–261.
Chopra, S., Motwani, S.K., Iqbal, Z., Talegaonkar, S., Ahmad, F.J., Khar, R.K., 2007. Optimisation of polyherbal gels for vaginal drug delivery by Box-Behnken statistical design. Eur. J. Pharm. Biopharm. 67, 120–131.
Chougule, M.B., Padhi, B.K., Jinturkar, K.A., Misra, A., 2007. Development of dry powder inhalers. Recent Pat. Drug Deliv. Formul. 1, 11–21.
Corrigan, D.O., Corrigan, O.I., Healy, A.M., 2004. Predicting the physical state of spray dried composites: salbutamol sulphate/lactose and salbutamol sulphate/polyethylene glycol co-spray dried systems. Int. J. Pharm. 273, 171–182.
Corrigan, D.O., Corrigan, O.I., Healy, A.M., 2006. Physicochemical and in vitro deposition properties of salbutamol sulphate/ipratropium bromide and salbutamol sulphate/excipient spray dried mixtures for use in dry powder inhalers. Int. J. Pharm. 322, 22–30.
Costantino, H.R., Andya, J.D., Nguyen, P.A., Dasovich, N., Sweeney, T.D., Shire, S.J., Hsu, C.C., Maa, Y.F., 1998. Effect of mannitol crystallization on the stability and aerosol performance of a spray-dried pharmaceutical protein, recombinant humanized anti-IgE monoclonal antibody. J. Pharm. Sci. 87, 1406–1411.
de Lange, E.C., de Boer, A.G., Breimer, D.D., 2000. Methodological issues in microdialysis sampling for pharmacokinetic studies. Adv. Drug Deliv. Rev. 45, 125–148.
Eisenberg, E.J., Conzentino, P., Eickhoff, W.M., Cundy, K.C., 1993. Pharmacokinetic measurement of drugs in lung epithelial lining fluid by microdialysis: aminoglycoside antibiotics in rat bronchi. J. Pharmacol. Toxicol. Methods 29, 93–98.
El-Gendy, N., Berkland, C., 2009. Combination chemotherapeutic dry powder aerosols via controlled nanoparticle agglomeration. Pharm. Res. 26, 1752–1763.
Giovagnoli, S., Blasi, P., Schoubben, A., Rossi, C., Ricci, M., 2007. Preparation of large porous biodegradable microspheres by using a simple double-emulsion method for capreomycin sulfate pulmonary delivery. Int. J. Pharm. 333, 103–111.
Gliniski, J., Chavepeyer, G., Platten, J.K., 2000. Surface properties of aqueous solutions of L-leucine. Biophys. Chem. 84, 99–103.
Goldman, J.M., Bayston, S.M., O'Connor, S., Meigh, R.E., 1990. Inhaled micronised gentamicin powder: a new delivery system. Thorax 45, 939–940.
Hagerman, J.K., Hancock, K.E., Klepser, M.E., 2006. Aerosolised antibiotics: a critical appraisal of their use. Expert Opin. Drug Deliv. 3, 71–86.
Hall, I.H., Schwab, U.E., Ward, E.S., Butts, J.D., Wolford, E.T., Ives, T.J., 2002. Disposition and intracellular activity of azithromycin in human THP-1 acute monocytes. Int. J. Antimicrob. Agents 20, 348–360.
Hand, W.L., Hand, D.L., 2001. Characteristics and mechanisms of azithromycin accumulation and efflux in human polymorphonuclear leukocytes. Int. J. Antimicrob. Agents 18, 419–425.
Herkner, H., Muller, M.R., Kreischitz, N., Mayer, B.X., Frossard, M., Joukhadar, C., Klein, N., Lackner, E., Muller, M., 2002. Closed-chest microdialysis to measure antibiotic penetration into human lung tissue. Am. J. Respir. Crit. Care Med. 165, 273–276.
Hoepelman, I.M., Schneider, M.M., 1995. Azithromycin: the first of the tissue-selective azalides. Int. J. Antimicrob. Agents 5, 145–167.
Islam, N., Gladki, E., 2008. Dry powder inhalers (DPIs) – a review of device reliability and innovation. Int. J. Pharm. 360, 1–11.
Joukhadar, C., Derendorf, H., Muller, M., 2001a. Microdialysis. A novel tool for clinical studies of anti-infective agents. Eur. J. Clin. Pharmacol. 57, 211–219.
Joukhadar, C., Frossard, M., Mayer, B.X., Brunner, M., Klein, N., Siostrzonek, P., Eichler, H.G., Muller, M., 2001b. Impaired target site penetration of beta-lactams may account for therapeutic failure in patients with septic shock. Crit. Care Med. 29, 385–391.
Kasahara, K., Maeda, K., Mikasa, K., Uno, K., Takahashi, K., Konishi, M., Yoshimoto, E., Murakawa, K., Kita, E., Kimura, H., 2004. Longterm azithromycin therapy for three patients with chronic lower respiratory tract infections. J. Infect. Chemother. 10, 42–45.
Kohlhammer, Y., Raspe, H., Marre, R., Suttorp, N., Welte, T., Schafer, T., 2007. Antibiotic treatment of community acquired pneumonia varies widely across Germany. J. Infect. 54, 446–453.
Kollef, M.H., 2005. The importance of antimicrobial resistance in hospital-acquired and ventilator-associated pneumonia. Curr. Anaesth. Crit. Care 16, 209–219.
Learoyd, T.P., Burrows, J.L., French, E., Seville, P.C., 2008. Chitosan-based spray-dried respirable powders for sustained delivery of terbutaline sulfate. Eur. J. Pharm. Biopharm. 68, 224–234.

Li, F.Q., Hu, J.H., Lu, B., Yao, H., Zhang, W.G., 2001. Ciprofloxacin-loaded bovine serum albumin microspheres: preparation and drug-release in vitro. J. Microencapsul. 18, 825–829.
Li, H.Y., Neill, H., Innocent, R., Seville, P., Williamson, I., Birchall, J.C., 2003. Enhanced dispersibility and deposition of spray-dried powders for pulmonary gene therapy. J. Drug Target. 11, 425–432.
Li, H.Y., Seville, P.C., Williamson, I.J., Birchall, J.C., 2005. The use of amino acids to enhance the aerosolisation of spray-dried powders for pulmonary gene therapy. J. Gene Med. 7, 343–353.
Li, J., Nie, S., Yang, X., Wang, C., Cui, S., Pan, W., 2008. Optimization of tocol emulsions for the intravenous delivery of clarithromycin. Int. J. Pharm. 356, 282–290.
Mitchell, M.A., 2005. Azithromycin. In: Seminars in Avian and Exotic Pet Medicine, 14, pp. 212–214.
Mosen, K., Backstrom, K., Thalberg, K., Schaefer, T., Kristensen, H.G., Axelsson, A., 2004. Particle formation and capture during spray drying of inhalable particles. Pharm. Dev. Technol. 9, 409–417.
Nicasio, A.M., Eagye, K.J., Nicolau, D.P., Shore, E., Palter, M., Pepe, J., Kuti, J.L., 2009. Pharmacodynamic-based clinical pathway for empiric antibiotic choice in patients with ventilator-associated pneumonia. J. Crit. Care.
O'Doherty, B., Muller, O., 1998. Randomized, multicentre study of the efficacy and tolerance of azithromycin versus clarithromycin in the treatment of adults with mild to moderate community-acquired pneumonia. Azithromycin Study Group. Eur. J. Clin. Microbiol. Infect. Dis. 17, 828–833.
Ohashi, K., Kabasawa, T., Ozeki, T., Okada, H., 2009. One-step preparation of rifampicin/poly(lactic-co-glycolic acid) nanoparticle-containing mannitol microspheres using a four-fluid nozzle spray drier for inhalation therapy of tuberculosis. J. Control. Release 135, 19–24.
Pai, V.B., Nahata, M.C., 2001. Efficacy and safety of aerosolized tobramycin in cystic fibrosis. Pediatr. Pulmonol. 32, 314–327.
Pilcer, G., Sebti, T., Amighi, K., 2006. Formulation and characterization of lipid-coated tobramycin particles for dry powder inhalation. Pharm. Res. 23, 931–940.
Rabbani, N.R., Seville, P.C., 2005. The influence of formulation components on the aerosolisation properties of spray-dried powders. J. Control. Release 110, 130–140.
Rennard, S.I., Basset, G., Lecossier, D., O'Donnell, K.M., Pinkston, P., Martin, P.G., Crystal, R.G., 1986. Estimation of volume of epithelial lining fluid recovered by lavage using urea as marker of dilution. J. Appl. Physiol. 60, 532.
Schanker, L.S., Less, M.J., 1977. Lung pH and pulmonary absorption of nonvolatile drugs in the rat. Drug Metab. Dispos. 5, 174–178.
Seville, P.C., Learoyd, T.P., Li, H.Y., Williamson, I.J., Birchall, J.C., 2007. Amino acid-modified spray-dried powders with enhanced aerosolisation properties for pulmonary drug delivery. Powder Technol. 178, 40–50.
Son, Y.J., McConville, J.T., 2008. Advancements in dry powder delivery to the lung. Drug Dev. Ind. Pharm. 34, 948–959.
Steckel, H., Brandes, H.G., 2004. A novel spray-drying technique to produce low density particles for pulmonary delivery. Int. J. Pharm. 278, 187–195.
Todo, H., Iida, K., Okamoto, H., Danjo, K., 2003. Improvement of insulin absorption from intratracheally administrated dry powder prepared by supercritical carbon dioxide process. J. Pharm. Sci. 92, 2475–2486.
Twiss, J., Byrnes, C., Johnson, R., Holland, D., 2005. Nebulised gentamicin-suitable for childhood bronchiectasis. Int. J. Pharm. 295, 113–119.
Ventura, C.A., Tommasini, S., Crupi, E., Giannone, I., Cardile, V., Musumeci, T., Puglisi, G., 2008. Chitosan microspheres for intrapulmonary administration of moxifloxacin: interaction with biomembrane models and in vitro permeation studies. Eur. J. Pharm. Biopharm. 68, 235–244.
Wu, J.W., Shih, H.H., Wang, S.C., Tsai, T.H., 2004. Determination and pharmacokinetic profile of pyrazinamide in rat blood, brain and bile using microdialysis coupled with. Anal. Chim. Acta 522, 231–239.
Young, P.M., Cocconi, D., Colombo, P., Bettini, R., Price, R., Steele, D.F., Tobyn, M.J., 2002. Characterization of a surface modified dry powder inhalation carrier prepared by "particle smoothing". J. Pharm. Pharmacol. 54, 1339–1344.
Zeitlinger, M., Müller, M., Joukhadar, C., 2005. Lung microdialysis—a powerful tool for the determination of exogenous and endogenous compounds in the lower respiratory tract (mini-review). AAPS J. 7, 600–608.
Zeng, X.M., Martin, G.P., Tee, S.K., Marriott, C., 1998. The role of fine particle lactose on the dispersion and deaggregation of salbutamol sulphate in an air stream in vitro. Int. J. Pharm. 176, 99–110.
Zhang, Y., Liu, X., Cui, Y., Huang, H., Chi, N., Tang, X., 2009. Aspects of degradation kinetics of azithromycin in aqueous solution. Chromatographia 70, 67–73.
Zhao, M., You, Y., Ren, Y., Zhang, Y., Tang, X., 2008. Formulation, characteristics and aerosolization performance of azithromycin DPI prepared by spray-drying. Powder Technol. 187, 214–221.