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Development of inhalable formulations of anti-inflammatory drugs to potentially treat smoke inhalation injury in burn victims

A. Thai, J. Xiao, A.J. Ammit, R. Rohanizadeh*

Advanced Drug Delivery Group, Faculty of Pharmacy (A15), University of Sydney, Sydney, NSW, 2006, Australia

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ABSTRACT

Injury arising from smoke inhalation is a significant mortality risk in severe burned patients. Inflammatory processes are major contributors to the development of respiratory insufficiency owing to pulmonary oedema, formation of airway fibrin clots and hypoxaemia. Anti-inflammatory and anti-coagulant drugs such as heparin and pentoxifylline are currently systemically administered for the treatment of smoke inhalation. Delivery of these drugs in the form of inhalable particles could be an effective manner to achieve rapid targeted action for acceleration of the treatment. The study developed and characterised a series of spray-dried heparin and pentoxifylline dry powder formulations suitable for inhalation administration. Drug particles were co-spray-dried with leucine in varying ratios. Particle size analysis confirmed all powders (except 2%, w/w, pentoxifylline with 1%, w/w, leucine in spray-drying feed solution) had particle size in the optimal range ($\leq$5 μm) for deep lung drug deposition. Leucine supplementation dramatically altered heparin surface topography while pentoxifylline formulations were a mixture of elongated needles interspersed with wrinkly particles. Addition of leucine improved fine particle fraction of heparin and pentoxifylline. The study indicated manufacture of inhalable heparin and pentoxifylline was feasible and can potentially be an attractive delivery alternative to the more conventional systemic delivery route.

1. Introduction

1.1. Smoke inhalation injury

Smoke inhalation injury is a major contributor to morbidity and mortality in severely burned patients, in which risk for development of fatal respiratory failure is greater than 50% (Cetin et al., 2003). The co-presence of bronchopulmonary injury with cutaneous burns covering at least 30% of total body surface area increases mortality rate to more than 70% (Herndon et al., 1988; Mlcak et al., 2007). Inhalation injury may further predispose burned victims to pneumonia, which alone increases the mortality risk by 90% (Mlcak et al., 2007). The pathophysiologic mechanism underlying smoke inhalation injury is complex (Mccall and Cahill, 2005; Bidani et al., 1996; Tasaki et al., 2002). Thermal injury caused by inhalation of hot gases is mostly limited to the upper respiratory tract since the low heat capacity of dry air, reflex adduction of the vocal cords, and efficient heat-dissipating of the upper airways, all protect lower lung areas from heat injury (Cetin et al., 2003; Mlcak et al., 2007). However, inhalation of toxic products of chemical combustion and particulate constituents of smoke trigger the inflammatory cascade in the lower lung areas. Inflammatory processes are primarily regulated by inflammatory mediators released from damaged tissues and epithelial cells, including; (1) polymorphonuclear leukocytes (PML), (2) resident alveolar macrophages (AM), mast cells and trapped neutrophils, and (3) systemic interleukins (IL)-1, IL-6, IL-8 and tumour necrosis factor alpha (TNF-α) (Bidani et al., 1996; Cetin et al., 2003; Holt et al., 2008; Mlcak et al., 2007). Studies have shown acute respiratory insufficiency and inflammatory-mediated complications to be responsible for the most smoke inhalation injury-related deaths observed within 24–48 h post-fire (Cetin et al., 2003). Subsequent surfactant loss leads to alveolar collapse (atelectasis) and destruction of mucociliary transport, inhibiting bacterial clearance and increases the infection risk. Ulceration, extensive necrosis, and subsequent sloughing of the tracheobronchial mucosa further predispose the burns patient to secondary bacterial invasion and development of pneumonia following smoke inhalation (Herndon et al., 1988; Mccall and Cahill, 2005; Mlcak et al., 2007).

1.2. Current treatments for smoke inhalation injury

Because airway swelling and obstruction predominates early after smoke inhalation (within 72 h), therapies for prevention of

* Corresponding author. Tel.: +61 2 9351 2355; fax: +61 2 9351 4391.
E-mail address: raminr@pharm.usyd.edu.au (R. Rohanizadeh).

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airway collapse and associated hypoxia are critical. Mechanical intubation, ventilatory support, fluid resuscitation, and bronchial lavage are all strategies currently employed in the management of smoke-induced injury (Desai et al., 1998; Herndon et al., 1988; Hussein et al., 1988; Lv et al., 2006; Mccall and Cahill, 2005; Mlcak et al., 2007). The timely implementation of these strategies is particularly crucial, especially when airway integrity is compromised by injury. These medical interventions solely provide supportive therapies rather than treatment. Pharmacological agents are thus needed to launch an assault on the underlying pathogenesis of smoke inhalation injury, including inflammation of the lungs. Previous studies have investigated the anti-inflammatory and anticoagulant benefits of heparin and pentoxifylline in treating smoke inhalation injury. Heparin can improve acute lung injury by preventing fibrin clot formation to ease airway occlusion, hypoxia, atelectasis, and barotraumas. Idell et al. (1994) suggested that injury of tracheobronchial epithelial cells activates the extrinsic pathway of coagulation, in which epithelium sloughing can combine with a protein-rich exudate to produce airway casts and fibrin clots (Murakami et al., 2003; Enkhbaatar et al., 2007b, 2008; Idell et al., 1994; Murakami et al., 2002). Thrombin is a key procoagulant and pro-inflammatory protease to activate fibrinogen and therefore forming fibrin in the final step of haemostasis. It has been reported that heparin enhances the thrombin inhibition ability of anti-thrombin III by indirect inactivation of Factor Xa (Murakami et al., 2003; Enkhbaatar et al., 2007a). Heparin is also an anionic compound, which can limit the microvascular endothelial permeability of cationic proteases (e.g., elastase) released by PML cells (Mlcak et al., 2001; Cox et al., 1993; Desai et al., 1998). In an ovine model of acute respiratory distress syndrome, Mlcak et al. (2001) showed that smoke-induced injury animals treated with nebulised heparin had significantly higher pulmonary compliance and lower airway resistance, compared to those treated with nebulised saline. Using a similar ovine model, Cox et al. (1993) further reported that intravenously administered heparin improved blood oxygenation and minimised barotraumas following severe smoke inhalation injury by decreasing tracheobronchial cast formation and pulmonary oedema. Clinical studies involving human subjects proved aerosolised heparin and N-acetylcysteine combinations to be effective (Mlcak et al., 2007; Holt et al., 2008; Desai et al., 1998). It has been reported that heparin treatment decreased fibrin cast formation, peak inspiratory pressures, and incidence of atelectasis, therefore, reducing the mortality rates, need for ventilatory support, and incidence of pneumonia (Mlcak et al., 2007; Holt et al., 2008; Desai et al., 1998). A standard treatment using nebulised heparin for patients with inhalation injury might include 5000–10,000 units of heparin and 3 mL normal saline nebulised every 4 h, alternating with 3–5 mL of 20% N-acetylcysteine for 7 days. This insures that the patient receives an aerosolised treatment every 2 h (Herndon, 2002).

Like heparin, pentoxifylline has demonstrated some benefits in improving lung function after smoke inhalation. 20 mg/kg intravenous injection of pentoxifylline [3,7-dimethyl-1-(5-oxohexyl)xanthine] followed by continuous infusion of pentoxifylline (2 mg/kg/h) has been shown to improve lung function in a rat model of smoke inhalation injury as described by a reduction in lung oedema, endothelial cell injury, neutrophil migration, magnitude of cytokine response and hypoxaemia (Ogura et al., 1994). *In vitro* studies exploring the effects of pentoxifylline in smoke inhalation injury demonstrated alteration of the intracellular signaling pathway responsible for gene transcription. Post-injury, the production and release of pro-inflammatory mediators such as TNF-α is heavily involved in host defence mechanisms and inflammation (Oliveira et al., 2003; Strieter et al., 1988). Oliveira et al. (2003) suggested pentoxifylline down-regulated TNF-α mRNA expression in rats during acute lung injury, while Strieter et al. (1988) advocated pentoxifylline inhibited endotoxin-mediated transcription of the TNF-α gene in peritoneal murine AMs, thereby hindering TNF-α, IL-1 and PAF activity. Furthermore, pentoxifylline has been well purported to inhibit cyclic AMP phosphodiesterase, thereby prolonging cellular cyclic AMP activity and delaying its inactivation (Ogura et al., 1994; Hussein et al., 1988). Other benefits of pentoxifylline treatment appear to stem from effects on microvascular circulation, which is a result of improved erythrocyte and leukocyte deformability, decreased plasma fibrinogen, and inhibition of platelet aggregation. This improves capillary blood flow and thereby tissue oxygenation (Ogura et al., 1994).

In an ovine model study by Tasaki et al. (2002) the authors compared the effects of combining heparin and lisofylline (a precursor which is converted to pentoxifylline in the liver) to either drug alone and showed that the combination of these two drugs enhanced the effects of these drugs alone in reducing the need for mechanical ventilation. It may be extrapolated that pentoxifylline should have a similar effect to lisofylline when used in combination with heparin, although further research may be required to validate this hypothesis.

1.3. Pulmonary drug delivery systems

Considering the localisation of smoke inhalation injury in deep lung areas, pulmonary delivery of heparin and pentoxifylline particles may accelerate the healing of lung injuries by providing rapid local onset of action and allowing deep penetration into the lungs (Rabbani and Seville, 2005; Smyth, 2003). Pulmonary delivery enables the deposition of therapeutic drug concentrations at the target site without the need to administer large doses. In systemic administration, large doses must be administered to achieve significant drug uptake by the lungs bronchiolar and alveolar regions, resulting in greater incidence of side effects (Tasaki et al., 2002; Adi et al., 2008b; Sham et al., 2004; Qi et al., 2004; Smyth, 2003). Both nebuliser and dry powder inhaler (DPI) systems can be used to achieve target deposition in the deep lung areas. Comparatively, DPI formulations are more portable and cost-effective than nebulisers, which require routine maintenance, increased administration time, and generally have low drug reproducibility. DPIs also do not rely on propellant technology for drug aerosolisation and necessitate synchoronization of inhalation with device actuation akin to pressurised metered dose inhalers (pMDIs). Powder dispersion in DPI is driven by patient inspiration, in which efficiency is dictated by formulation and device design. Dry powders also confer enhanced stability during storage since there is less susceptibility to chemical degradation in the solid state (Rabbani and Seville, 2005; Adi et al., 2008a; Li et al., 2003, 2005; Finlay, 2001).

The present study aimed to develop and characterise inhalable aerosol formulations of heparin and pentoxifylline particles. There are no inhalable heparin or pentoxifylline dry powder products clinically available. *In vitro* characterisation of nebulised heparin generated by jet and ultrasonic nebulisers demonstrated the inefficiency of nebulisation. Of a loading dose of 80,000 IU of heparin, 45,000 IU remained in the dead space of the nebuliser; 20,000 IU deposited on the exhalation filter, while 15,000 IU was captured on the inhalation filter, which corresponds to a respirable mass of 10,000 IU of heparin (Bendstrup et al., 1999). Therefore drugs in the form of inhalable solid aerosols may be able to deposit deeper and more efficiently in the lung, reaching damaged areas where nebulised drugs cannot. A solid aerosol may be particularly advantageous in delivery of drugs to the lungs as it allows a more controllable and prolonged dosage to be administered.

2. Materials and methods

2.1. Materials

Water-soluble lyophilized heparin sodium from porcine intestinal mucosa was supplied by Celsus Laboratories Inc. (Cincinnati, OH, USA). Pentoxifylline was obtained from MP Biomedicals LLC (Solon, OH, USA). L-Leucine was supplied from ICN Biomedicals Inc. (Aurora, OH, USA).

2.2. Preparation of powder formulations using spray-drying

A series of drug (heparin and pentoxifylline) powder formulations containing various concentrations of leucine were prepared by dissolving them in purified water and spray-drying the solution using a Büchi B-290 Mini spray-drier (Büchi, Switzerland). Leucine was added to the formulation to decrease the surface tension of pentoxifylline and heparin droplets during spray-drying, and therefore to aid dispersion of particles. Drug-to-leucine ratios of 1:1, 1:2 and 2:1 were investigated and for simplification sake, annotated in this study as follows, for example 1H:1L signifies 1% (w/w) heparin and 1% (w/w) leucine in the spray-drying feed solution. The drugs and leucine alone were also spray-dried as single agents using the same vehicle and conditions. A pilot study conducted in our research team has previously optimised the conditions for spray-drying of heparin and pentoxifylline based on the median particle size of prepared powder and the yield of spray-drying. Spray-drying feed solutions were prepared by solubilising leucine in water, heated at 90 °C for 20 min, followed by cooling to room temperature prior to addition of a pre-determined amount of active drug (heparin or pentoxifylline). Spray-drying conditions employed were as follows: inlet temperature 160 °C, outlet temperature 85–90 °C, airflow 600 L/min and solution feed rate 6 mL/min. Percentage yields of each powder following spray-drying were calculated. To minimise the effect of air humidity, immediately after spray-drying the prepared powders samples stored in sealed containers at room temperature and in a dessicator filled with silica beads.

2.3. Physicochemical characterisation of powder formulations

2.3.1. Particle size analysis

The particle size distribution of each spray-dried sample was evaluated by laser diffraction (Mastersizer 2000; Malvern Instruments Ltd.) using the Scirocco dry dispersion unit. Approximately 10 mg of each sample was analysed at a feed air pressure of 4 bars and feed rate of 100% over a 5 s time interval. A refractive index of 1.55 was used for all samples with obscuration limits set between 0.3 and 10%. All samples were measured in triplicate and the plots were presented as mean values.

2.3.2. Scanning electron microscopy (SEM)

The surface morphology and texture of particles in each powder formulation was qualitatively evaluated using field emission scanning electron microscopy (Zeiss UltraPlus Field Emission Scanning Electron Microscope), working at 8 kV. Prior to SEM observation, samples were mounted onto carbon double-adhesive tape and sputter-coated with gold (~15 nm thickness).

2.3.3. Quantitative dose uniformity testing

The powder homogeneity and percentage composition of each drug in the series of heparin and pentoxifylline formulations co-spray-dried with leucine were analysed using UV spectrophotometry (Hitachi U2000 Double Beam UV/Vis Spectrophotometer). Five samples were taken in a random manner from both pure dry-blended and spray-dried powders and dissolved in purified water for assay at wavelengths of 265 and 273.5 nm for heparin-containing and pentoxifylline-containing powders, respectively. These wavelengths were selected by performing wavelength scans using the same apparatus and were representative of wavelengths at which heparin and pentoxifylline both exhibit maximum absorbance. Results were expressed as a percentage of the nominal ratio.

2.3.4. In vitro aerosol performance studies

While the dry powder feeder of laser diffraction may indicate relative aerosol performance based on particle size, it does not reflect powder dispersion behaviour from a patient-actuated inhaler device. *In vitro* characterisation of aerosols is useful to predict the amount of fine particles available upon inhalation. The aerosolisation properties of the spray-dried powders were investigated using a multi-stage liquid impinger (MSLI) (Copley Scientific, Nottingham, UK). The design of this cascade impactor apparatus reflects the likely regional lung deposition of particles based on their aerodynamic diameter (Mitchell and Nagel, 2003). At a flow rate of 60 L/min (estimated to be equivalent to a deep inhalation manoeuvre when using a dry powder inhaler), the aerodynamic cut-off diameters of stages 1, 2, 3 and 4 are 13, 6.8, 3.1 and 1.7 μm, respectively. Particles with diameter size less than 1.7 μm were captured on a filter membrane. The flow rate through the MSLI was initially established to 60 L/min using a calibrated flow meter (TSI Model 4040C; TSI Instruments Ltd., Buckinghamshire, UK) and subsequently controlled by a rotary vein pump and solenoid valve timer.

Prior to testing, stages 1–4 were pre-loaded with purified water to solubilise the mass of powder anticipated to be deposited on the stages. For each spray-dried powder formulation, 25 mg of powder was manually filled into four size 3 hydroxypropyl methylcellulose (HPMC) capsules (Capsugel, Sydney, Australia) and each capsule consecutively placed into an Aeroliser® DPI device (Schering Corporation). The Aeroliser® device was inserted into a mouthpiece adaptor which was connected to the MSLI via a stainless steel throat. Once pierced, the liberated powder is drawn from the capsule through the MSLI for 4 s and the procedure repeated for the three remaining capsules containing the same powder before the mass of drug deposited in the throat, device, capsule and adaptor were separately solubilised in a suitable volume of water and samples taken from the stages for UV assay. Each spray-dried powder was tested using the MSLI procedure in triplicate.

Quantification of the recovered aqueous samples from the MSLI was analysed using UV spectrophotometry. Linear calibration curves were derived using the spray-dried powders to account for any possible interference from the spray-drying process or leucine addition. Although particle size is a good predictor of aerosol performance, fine particle dose is another important indicator for consideration. This fraction represents the mass fraction of drug particles with a mass median aerodynamic diameter smaller than 5 μm in the aerosol cloud. The complete *in vitro* aerosol performance profile for each drug formulation was represented as follows: total dose (TD) recovered from all stages of the MSLI, device and capsule; emitted dose (ED) recovered from all stages of the MSLI; fine particle dose (FPD) as the amount of drug recovered from stages 3 (3.1 μm), 4 (1.7 μm), and filter (<1.7 μm); fine particle fraction (FPF_{TD}) equivalent to FPD as a percentage of total dose recovered; fine particle fraction (FPF_{ED}) equivalent to FPD as a percentage of emitted dose; and amount of drug deposited on each stage of the MSLI.

2.3.5. In vitro cell toxicity of powder formulations

The cell toxicity of the spray-dried powder formulations was investigated using human alveolar basal epithelial A549 cell line, cultured in Dulbecco's Modified Eagles' Medium

(DMEM) supplemented with 2 mM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, 0.25 μg/mL amphotercin B, and 10% foetal bovine serum. Cells were grown in 10 cm dishes incubated in 5% CO_2 in air at 37 °C and were sub-cultured twice weekly. Prior to use in cell viability assays, the cells were seeded in 96-well plates at a density of 3200 cells/well (6 wells for each group) with 100 μL feeding media per well for 24 h. Prior to analysis, the spray-dried powders were sterilized using 25kGy gamma irradiation to reduce likelihood of contamination. In this particular type of assay, cell respiration was used as an indicator of cell viability, which was determined by the degree of mitochondrial-dependent reduction of 3-(3,4-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan crystals. To initiate the assay, the feeding media was replaced with 100 μL of fresh DMEM containing 1 mg/mL of different test powders. A positive control consisting of sodium dodecyl sulfate (SDS) in phosphate buffered saline PBS (0.5 mg/mL) and a negative control of culture media alone were used in the cell viability assay. Following 24 h of incubation, the plates were further incubated at 37 °C with 50 μL MTT (0.5 mg/mL) in non-sterile phosphate buffered saline (PBS) for 4 h. The medium was subsequently removed and replaced with 100 μL dimethyl sulfoxide (DMSO) which solubilised any synthesized formazan crystals. The absorbance (or optical density) of each well was measured using spectrophotometry (SpectraMax 190, Molecular Devices) at 550 nm. The Relative cell viability will be calculated using the following equation: Viability (%) = $A/C \times 100$, where A is the absorbance for each test formulation and C is the average absorbance for the negative control. The cytotoxicity of each spray-dried powder was tested in triplicate (six culture well/group for each culture).

3. Results

3.1. Spray-drying yield

The percentage yields of recovered powders from the spray dryer are shown in Fig. 1. Heparin-containing powders produced satisfactory recovery rates with yields ranging between 82 and 87%, whereas pentoxifylline-containing powders conveyed a gradual increase in yield from 30 to 50% with each 1% leucine addition to the formulation. However, when the pentoxifylline-to-leucine ratio was reversed to 2:1, the yield rose to 59%. Leucine spray-dried alone had a reasonable yield of 67%. Unlike heparin and leucine, attempts to spray-dry pentoxifylline alone were unsuccessful since it produced negligible yield (~0% yield). No further attempts were made to produce pentoxifylline alone.

Table 1
Mean particle size distributions (μm) obtained from laser diffraction (mean ± SD, $n = 3$).

Sample name	D_{10}	D_{50}	D_{90}
1H	0.65 ± 0.05	1.65 ± 0.04	3.91 ± 0.18
1H:1L	0.92 ± 0.03	3.19 ± 0.09	8.30 ± 0.14
1H:2L	1.11 ± 0.00	3.40 ± 0.02	7.94 ± 0.07
2H:1L	0.83 ± 0.01	2.96 ± 0.03	7.30 ± 0.08
1P:1L	0.88 ± 0.03	2.70 ± 0.13	8.12 ± 1.38
1P:2L	1.03 ± 0.00	3.02 ± 0.01	7.61 ± 0.02
2P:1L	3.24 ± 0.15	47.52 ± 1.29	273.60 ± 7.47
1L	0.84 ± 0.02	1.72 ± 0.03	3.59 ± 0.19

3.2. Particle size analysis

Fig. 2 demonstrates the particle size distributions of each of the heparin and pentoxifylline-containing powders. Combination powders containing both active drug and leucine produced similar unimodal particle size distributions falling within the size range for deep lung deposition (≤5 μm) with the exception of 2P:1L which exhibited variable polymodal distribution with a substantially greater median volume diameter of 47.52 ± 1.29 μm (Table 1). Spray-dried heparin or leucine as single agents displayed smaller particle size with median volume diameters of 1.65 ± 0.04 and 1.72 ± 0.03 μm, respectively (Table 1). Comparable median volume diameters of 3.19 ± 0.09, 3.40 ± 0.02 and 2.96 ± 0.03 μm were observed for heparin powders in the ratios 1:1, 1:2 and 2:1 with leucine, respectively (Table 1). Meanwhile, the median volume diameters for 1P:1L and 1P:2L were 2.70 ± 0.13 and 3.02 ± 0.01 μm, respectively (Table 1).

3.3. Scanning electron microscopy (SEM)

Scanning electron micrographs of the spray-dried powder formulations are shown in Fig. 3A–H. Spray-dried heparin particles exhibited a smooth spherical surface of approximately 1 μm in diameter (Fig. 3A). The combination of leucine with heparin significantly altered particle morphology (Fig. 3B–D). These

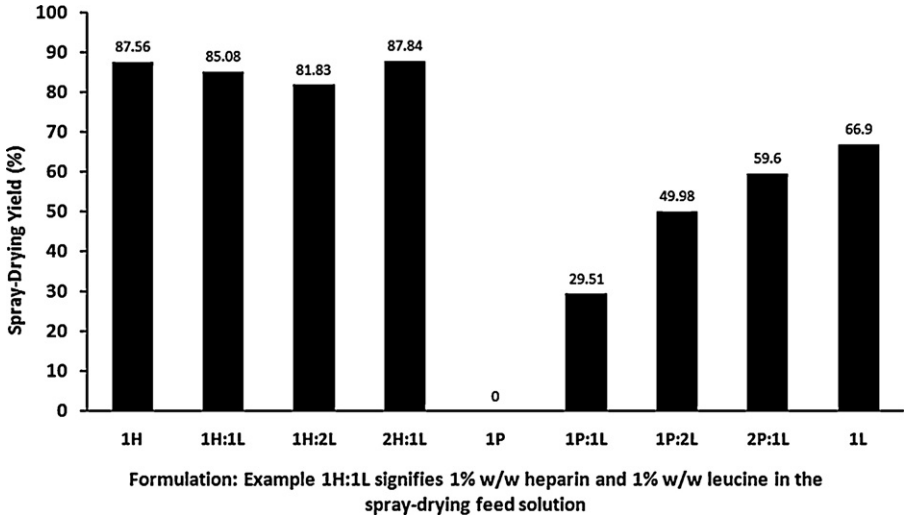

Fig. 1. Percentage yield of spray-drying for various powder formulations. Drug:leucine aqueous feed solutions consisting of leucine in varying ratios with heparin and pentoxifylline. For example 1H:2L signifies 1% (w/w) heparin and 2% (w/w) leucine in the spray-drying feed solution. Attempts to spray-dry pentoxifylline alone produced negligible yield (~0% yield).

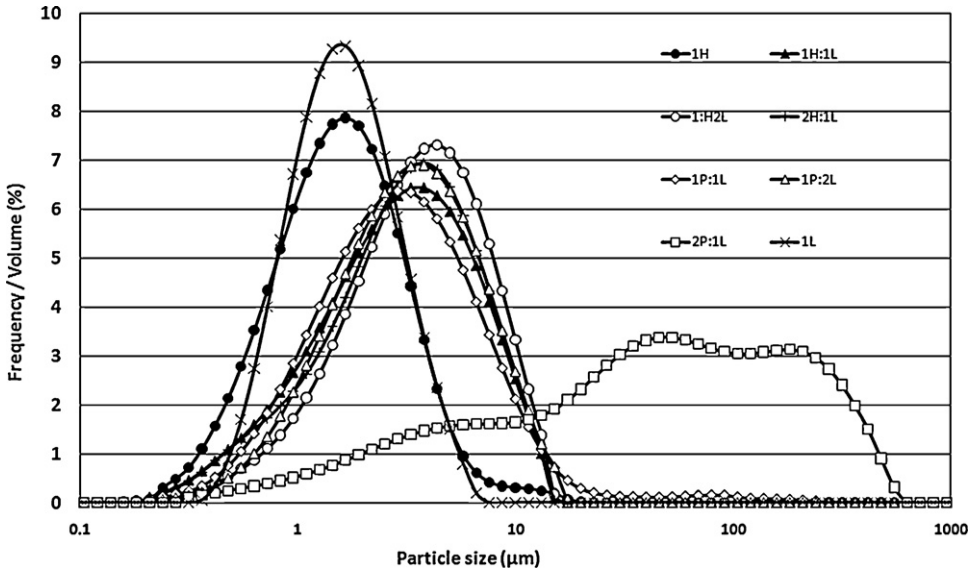

Fig. 2. Particle size distribution of different spray-dried powders measured using laser diffraction. The plots were presented as mean values of three measurements ($n = 3$) for each formulation.

spray-dried particulates exhibited a corrugated surface with partial particle collapse and cavity formation. Their particle sizes are in good agreement with those measured using laser diffraction. Large agglomerates consisting of particles with wrinkled topography interspersed with elongated particles are indicated in pentoxifylline-containing powders (Fig. 3E–G). The absence of these needle-like structures in leucine spray-dried particles (Fig. 3H) suggests that needle-like particles are representatives of pentoxifylline particles. The SEM micrograph for 2P:1L clearly supports the large particle size observation using laser diffraction, in which formation of a large aggregate failed to disperse in 4 bars of air pressure used in laser diffraction. The elongated needle shape of these particles may further contribute to the skewed nature of particle size distribution for 2P:1L group measured by laser diffraction. It should be noted that laser diffraction is not suitable for sizing elongated particles because the diffraction pattern cannot be accommodated by the algorithm from which the data is derived and serious error arise.

3.4. Quantitative dose uniformity testing

The amount of heparin and pentoxifylline in their respective spray-dried powders was calculated using UV spectrophotometry with standards prepared from pure purchased heparin and pentoxifylline powders. The results of uniformity test are illustrated in Fig. 4. Percentage deviations of greater than 100% from the theoretical expected drug-to-leucine ratio conveyed by all spray-dried powders, suggesting the drug-to-leucine ratios in spray-dried powders increased compared to the initial composition of the aqueous feed solution fed into the spray dryer. Significant differences ($p < 0.05$) were found for heparin-containing powders (Fig. 4).

3.5. In vitro aerosol performance studies

Percentage depositions of spray-dried heparin and pentoxifylline formulations on the various components and stages of the MSLI are shown in Fig. 5. Heparin alone exhibited poor aerosol performance with the majority of powder retained in the capsule and device (Fig. 5A). The lung deposition profile of heparin improved with leucine incorporation into the formulation. However increasing leucine content from 1 to 2% (w/w) (e.g., comparing 1H:1L and 1H:2L) did not significantly improve deposition in later stages (S3, S4 and filter) for better aerosol performance. Reversing this ratio to produce 2H:1L furthermore did not either enhance the amount of heparin deposited in the later stages. 1P:1L and 2P:1L both exhibit extensive powder accumulation in stage 1 while 1P:2L had more fair deposition across the stages of the MSLI (Fig. 5B). FPF_{TD} (fine particle fraction) equivalent to the FPD (fine particle dose) expressed as a percentage of the total dose recovered are shown in Fig. 6. Statistical analysis of the FPF_{TD} data suggests leucine adjunct imposed a positive effect on dispersion efficiency (one-way ANOVA test $p < 0.05$) for both heparin and pentoxifylline formulations. Based purely on FPF_{TD}, 1H:1L and 1P:2L conveyed best aerosol performance.

3.6. In vitro cell toxicity of spray-dried powder formulations

The viability of A549 lung epithelial cells following 24 h exposure to the spray-dried formulations is shown in Fig. 7. The viability of the untreated negative control was assigned 100% in which all other values were normalised with reference to this percentage. The positive control (not shown) consisting of sodium dodecyl sulfate, which lysed the cells, reduced mean viability to 23% relative to the untreated negative control. There were no significant differences observed for leucine itself and heparin-containing formulations however; this was not the case for pentoxifylline-containing powders (unpaired Student's t-test, $p < 0.05$). The cell viability of leucine was at 86% (absorbance 0.395), which is not statistically different to the untreated negative control, suggesting the pentoxifylline component of the P:L formulations may be responsible for the toxicity relative to the control under the conditions studied.

4. Discussion

4.1. Spray-dried yield

Spray-drying as a production method to manufacture dry powders for inhalation generated relatively good yields for heparin-containing formulations. High yield values are particularly desirable since this is an economic and practical consideration for the commercial manufacture of these drug particles. High mass recovery rates ranging between 82 and 87% were achieved for all heparin powders regardless of the heparin-to-leucine ratio (Fig. 1),

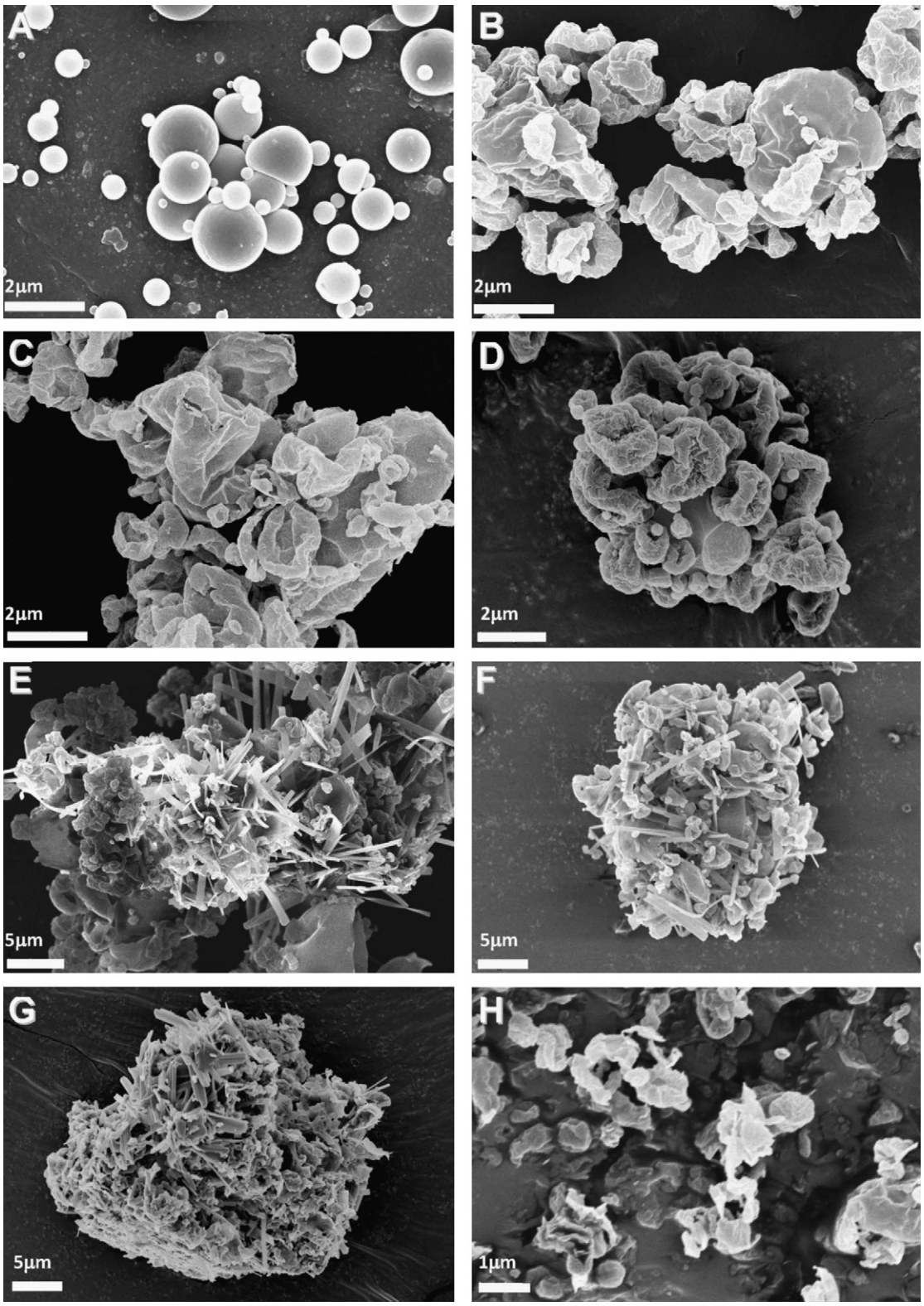

Fig. 3. Scanning electron micrographs of spray-dried (A) heparin; (B) 1% heparin and 1% leucine; (C) 1% heparin and 2% leucine; (D) 2% heparin and 1% leucine; (E) 1% pentoxifylline and 1% leucine; (F) 1% pentoxifylline and 2% leucine; (G) 2% pentoxifylline and 2% leucine; and (H) 1% leucine.

which implies leucine does not serve a significant role in increasing the yield of heparin. This was in contrast to pentoxifylline formulations which were prone to adhesion to the cyclone wall when leucine was not used. Since the cyclone precedes the powder collection vessel, powder accumulation narrows the neck of the cyclone over the time course of the spray-drying process to further hamper powder collection. Preliminary studies showed pentoxifylline spray-dried alone produced negligible yield, possibly due to the presence of electrostatic charge and van der Waals forces between pentoxifylline particles and the cyclone wall. The anti-adherent properties of leucine therefore enhanced yield by reducing surface charge interactions between these two interfaces, leading to higher

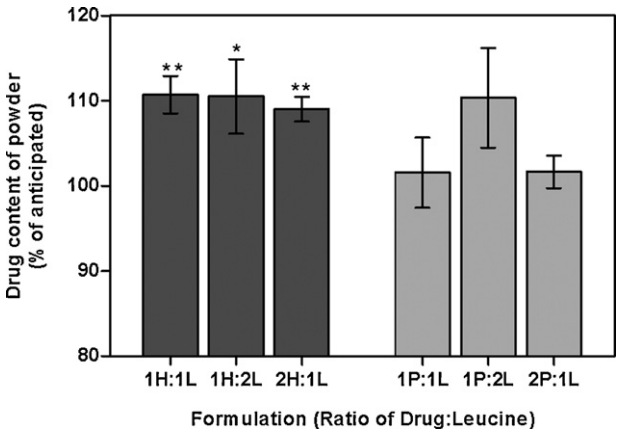

Fig. 4. Drug content of spray-dried powders expressed as a percentage of the drug-to-leucine ratio in the feed solution of spray dryer ($n = 5$). Symbols (*/**) denote significant difference (unpaired Student's t-test; $^{*}p < 0.05$; $^{**}p < 0.01$) between anticipated (based on drug% in the feed solution) and experimental concentrations of drugs in different spray-dried powders. Percentage deviations of greater than 100% from the theoretical expected drug-to-leucine ratio conveyed by all spray-dried powders.

spray-dried yields. The higher yield observed for 2P:1L relative to 1P:1L and 1P:2L may be explained by its overall larger particle size, which probably assisted its deposition from the cyclone into the collection vessel by way of gravity effects.

4.2. Particle size analysis

Particle size is an important determinant of successful delivery of dry powder aerosols to the lungs, which requires careful consideration of the powder production process, formulation, and inhaler device. The particle size distributions of each of the heparin or pentoxifylline powders are depicted in Fig. 2. Since inflammatory processes are more prevalent in deeper alveolar regions, drug deposition in the upper tracheobronchial region is not desirable and risk removal away from the lungs by mucociliary clearance (Sham et al., 2004). It should be noted that direct heat damage caused by fire is only limited to the larynx and vocal cords. What is more relevant in terms of smoke inhalation is the injury caused by poisonous gases, and not hot gases. Toxic chemicals released by combustion cause major damage to epithelial cells in the lung and endothelial cells in pulmonary capillaries. Since inhalation of toxic gases, thereby the subsequent inflammatory processes, are prevalent in deeper alveolar regions, it was therefore the goal of this study to fabricate inhalable drug particles capable to reach the deeper lung areas. An aerodynamic diameter of less than 5 μm is favourable to avoid deposition by inertial impaction and/or sedimentation in the oropharyngeal cavity and maximise deposition in peripheral lung areas. All spray-dried powders satisfied this criterion with the exception of 2P:1L. Furthermore, the consistent particle size distribution observed for all drug-to-leucine powder formulations except 2P:1L, demonstrating particle size was independent of the used drug-to-leucine ratio. This may correlate with the fact that all powders were spray-dried using the same conditions or the negligible physical effects of leucine on drug particle size.

The polymodal nature of the particle size distribution for 2P:1L (Fig. 2) can be accounted for by examining the morphology of these particles using SEM observation. As shown in Fig. 3, the spray-dried particles obtained from 2P:1L formulation demonstrated agglomerates of elongated needle-like structures among wrinkled particles (presumably leucine). When needle-like particles are dry-dispersed in laser diffraction particle size measurement, they may rotate at different angles, distorting volume diameter measurements to produce a variable particle size distribution. Size distribution for 2P:1L was far from the desired respirable size range, suggesting the drug powder is highly unlikely to reach alveolar regions where its action is desired.

Although such aforementioned agglomerates consisting of needle-like and wrinkled particles were also observed in SEM images of 1P:1L and 1P:2L formulations (Fig. 3E and F), their particle size distribution measured by laser diffraction was monomodal with highest size distribution around 5 μm. Despite similar morphologies conveyed in corresponding pentoxifylline–leucine powders, this contradiction might be due to the weak interfacial adhesion exhibited in 1P:1L and 1P:2L particles compared to those in 2P:1L group, resulting in breakdown of agglomerates to generate smaller particles by 4 bars air pressure during laser diffraction measurement.

4.3. Particle morphology

SEM images revealed smooth spherical heparin particles of small aerodynamic diameter (1 μm mean diameter). Of interest, the embedment of much smaller spheres on the surface of larger heparin particles suggested heparin particles are hollow in nature. Increased surface contact area arising from the spherical nature of heparin particles makes them more prone to cohesion, which translated to poor aerosolisation performance of heparin particles (without leucine) observed in this study. Nevertheless, when leucine is co-spray-dried with heparin, the surface of the particles became rough and corrugated (Fig. 4B–D). Partial particle collapse with cavity formation was also evident, which is in contrast with studies conducted by Shur et al. (2008) who produced smooth spherical heparin particles when co-spray-dried with leucine. Particle morphology is predominantly dictated by the selection of appropriate spray-drying conditions. The study by Shur et al. (2008) used the same inlet and measured outlet temperatures, however higher spray flow rates (700 L/h) employed compared to our experimental flow rate of 600 L/h may have affected the drying time of the heparin particles. In a spray-drier, slower air flow rates correspond to longer residence time in the spray dryer for the droplets to evaporate, thus reducing the drying efficiency simultaneously. Lingering heparin droplets with leucine in the spray dryer may therefore establish wrinkled morphology due to particle collapse before it deposits in the collection vessel. Surface corrugation may be beneficial in enhancing powder re-dispersion for inhalation. Furthermore, increasing leucine content from 1% (w/w) to 2% (w/w) did not appear to significantly alter particle morphology or tendency for agglomeration.

Spray-dried leucine alone comprised of hollow wrinkled microparticles, which has been reported by Najafabadi et al. (2004). The surfactant property of leucine allows it to migrate and enrich at the air–water interface of droplets such that during the course of rapid drying, this surface layer prevents the immediate evaporative removal of water from the interior of the particles. The resulting internal pressure accumulation eventually brings about particle collapse following complete removal of water to produce corrugated surface topography (Raula et al., 2007).

Fig. 3E and F featured aggregates of pentoxifylline and leucine particles with 1:1 and 1:2 ratios, respectively. The needle-like structures could be assigned as belonging to pentoxifylline since spray-dried leucine particles alone were wrinkled with irregular surface morphology (Fig. 3H). Previous studies investigating the spray-dried production of similar xanthine derivatives such as theophylline and caffeine also showed particles materialising as ensembles of small needles as observed for pentoxifylline in this study (Sacchetti et al., 2002; Asada et al., 2004). Moreover, it appeared that the two components of composite pentoxifylline-leucine formulations are not well incorporated to produce uniform particle morphology. The lack of amalgamation may be attributable

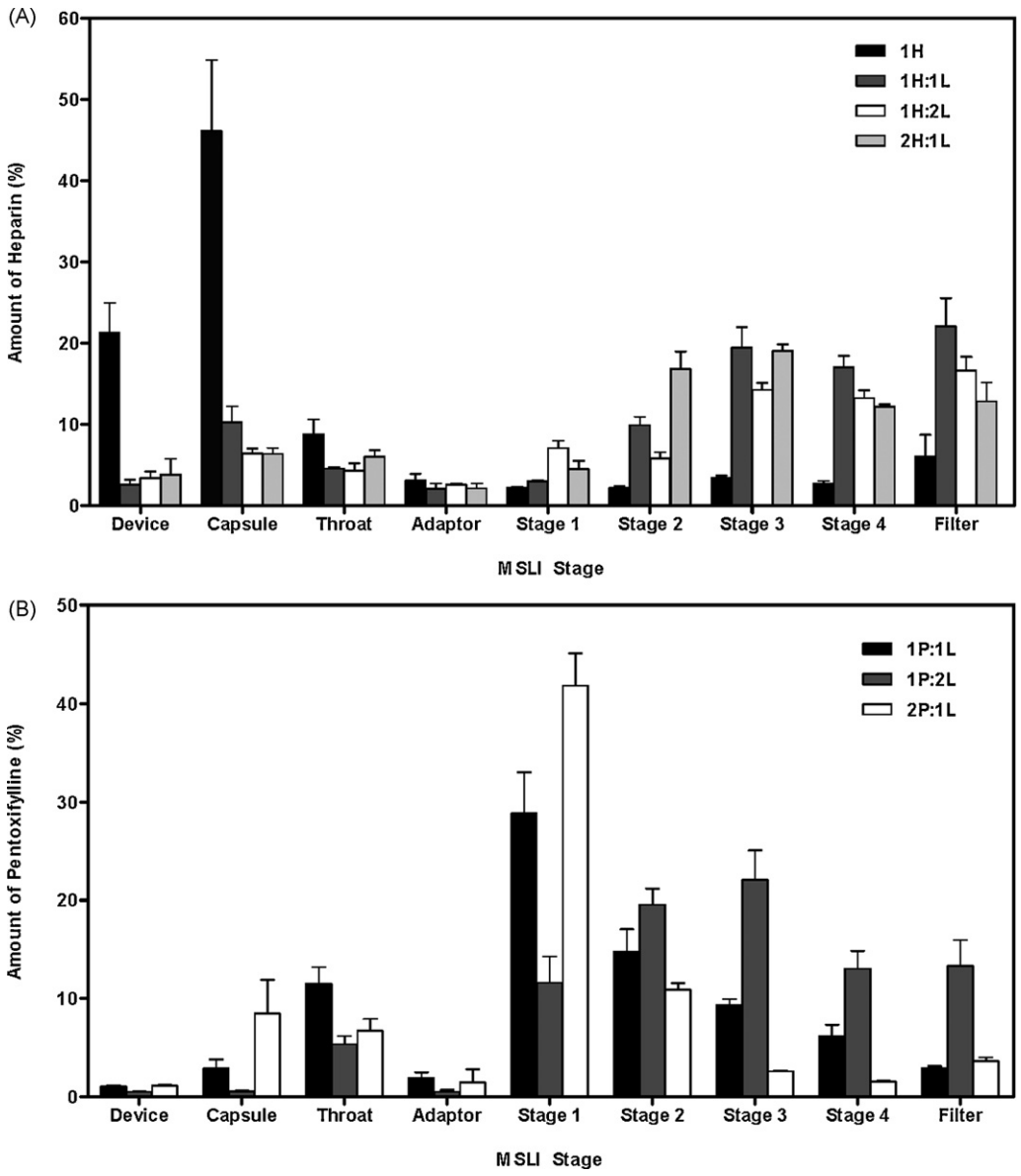

Fig. 5. Mean percentage drug depositions of (A) heparin, and (B) pentoxifylline in varying ratios with leucine in the device, capsule, throat, adaptor and each stage of the MSLI ($n = 3$).

to two possibilities: (1) leucine solution may not have been totally dissolved in pentoxifylline solution prior to spray-drying due to the different solubilities of pentoxifylline and leucine in water, or (2) formation of leucine and pentoxifylline solid phase (from liquid phase) may not have taken place concurrently in the spray dryer to produce integrated particles. Subsequent aggregation of needle-like pentoxifylline and wrinkly leucine particles therefore occurred following the drying process in the drying chamber. It is also possible that heparin–leucine formulations may be composed of separated heparin and leucine particles. However this cannot be established solely based on SEM images due to similar morphology between heparin and leucine particles. Overall, separation of drug and leucine components within the formulation has particular implications on powder homogeneity and ultimately dose reproducibility. Accurate dose metering of the powder is especially critical since DPI devices such as the Aeroliser® rely on capsules for unit dose loading.

Although spray-drying technique mostly results in formation of amorphous structure of drug powders (Chan and Chew, 2003), it is possible that such elongated needle-like structure of pentoxifylline could be a crystalline structure. In a pilot study, immediately visualised freshly spray-dried pentoxifylline with leucine using SEM confirmed crystalline structures despite a relatively short intermission between manufacturing and imaging. In this study, crystallisation could have been induced by absorption of atmospheric moisture arising from improper storage of powders. Whether or not spray-dried pentoxifylline particles have crystalline structure can be confirmed by conducting X-ray diffraction. The role of crystalline structure of particles in influencing aerosol powder dispersion and subsequently lung deposition is unclear.

4.4. Quantitative dose uniformity testing

The drug composition of the spray-dried powders relative to the amount initially dissolved in the aqueous spray-drying feed solution was tested in this study. Following pentuplicate analysis for each co-spray-dried formulation, mean percentage deviations of more than 100% suggested greater drug content relative to leucine in the spray-dried powder (Fig. 4). In particular, heparin-containing powders conveyed statistically significant deviations

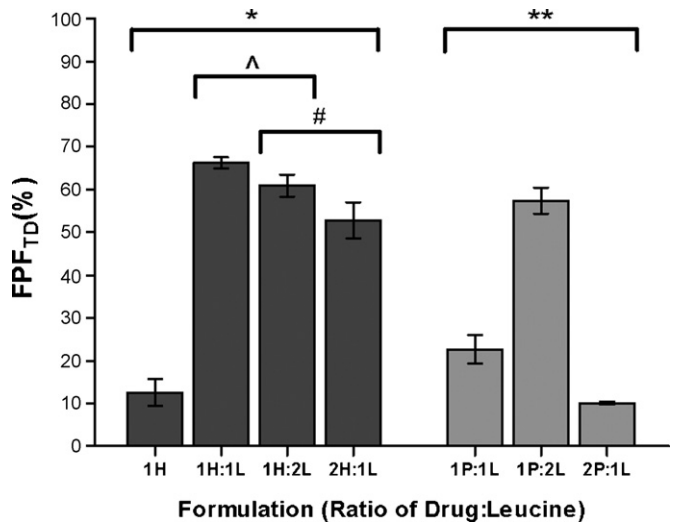

Fig. 6. Fine particle fractions (FPF) equivalent to the fine particle dose (FPD) expressed as a percentage of the total dose recovered for both heparin and pentoxifylline-containing powders ($n = 3$). Symbols (*/**) denote significant difference (ANOVA, $p < 0.05$; post hoc Tukey pair-wise) between FPFs of all pairs of heparin (*) and pentoxifylline (**) formulations except between 1H:1L vs. 1H:2L (^); and 1H:2L vs. 2H:1L (#).

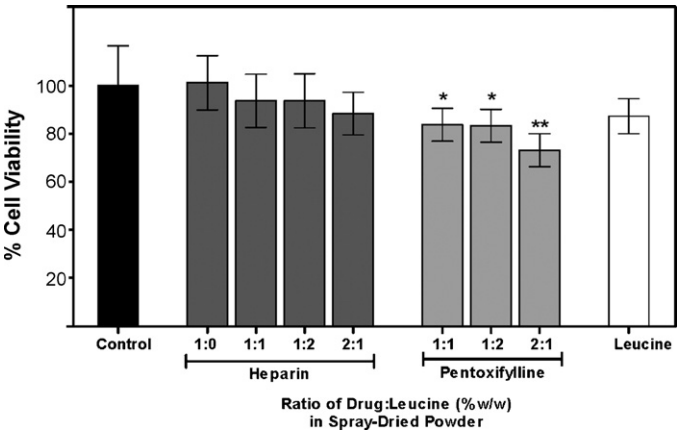

Fig. 7. The % of cell viability (compared to control) following 24 h exposure to powder formulations. Symbols (*/**) denote significant difference (unpaired Student's t-test ($n = 18$); *$p < 0.05$; **$p < 0.01$) between the cell viabilities of the formulation relative to the untreated control.

from the theoretical concentrations, insinuating non-uniform distribution of heparin throughout the formulation. As previously discussed, it is possible that heparin and leucine particles are not well incorporated to form single particles. Although it is not clear why spray-drying may have resulted in loss of leucine, it is feasible that due to the surfactant properties of leucine, the presence of a critical amount of leucine can instigate formation of micelle-like structures within which heparin particles are encapsulated by leucine. Nevertheless, unfractionated heparin has high molecular weights (MW) in the range 12,000–15,000 compared to leucine which has MW of 131.2. Considering the ratios of drug-to-leucine used, it is likely the relatively larger heparin molecules surround the smaller hydrophobic leucine molecules in the aqueous solution prior to spray-drying, resulting in encapsulation of leucine (Rabbani and Seville, 2005). The MW difference between pentoxifylline (MW, 278) and leucine is less significant, insinuating less potential for encapsulation of leucine by pentoxifylline; however, SEM images did appear to indicate separated leucine and pentoxifylline particles in pentoxifylline-leucine formulations. It is important to note based on our previous pilot study it was assumed in UV analysis that leucine did not interfere with absorbance readings of either heparin or pentoxifylline at wavelengths 265 and 273.5 nm, respectively. This assumption is attributable to limitation of UV spectroscopy, which cannot distinguish between absorbances of different excipients if they have similar peak wavelengths.

4.5. In vitro aerosol performance studies

Fine particle fraction (FPF_{TD}) can be used as a performance indicator for inhalation drug delivery. The FPF_{TD} is represented by the mass of drug recovered from stages 3, 4 and the filter, which reflects capture of particles with diameters below 5 μm. For each formulation, the amount of drug deposited on different stages of the MSLI was expressed as a percentage of the dose loaded into the capsules (Fig. 5) and the FPF_{TD} was calculated and plotted (Fig. 6). The full aerosolisation profile for each formulation is outlined in Table 2. Based purely on highest FPF_{TD} for heparin-containing formulations, 1H:1L produced the best performance. The poor dispersion profile of 1% heparin may be explained by the small particle size leading to cohesive particle behaviour.

Analysis of the *in vitro* data suggested enhanced deposition of heparin on later stages of the MSLI when leucine was included in the formulation. A dramatic improvement in FPF from 12.6 ± 3.1 to 64.5 ± 1.3% was observed when 1% leucine was added to 1% heparin and 59.9 ± 2.6% when 2% leucine was added. Notably there is no further advantage of increasing leucine content to maximum saturation at 2% (w/w) in spray-drying feed solution to optimise fine particle fraction of drug in the aerosol cloud. Based on Smith and Parry-Billings (2003) study, conventional dry powder formulations typically convey FPFs <30% at flow rates up to 90 L/min, therefore it is important to note that these leucine-supplemented formulations displayed relatively good dispersion characteristics overall regardless of the quantity of leucine added. Statistical analysis of the FPF data supported this observation that leucine adjunct engendered a significant positive effect on dispersive efficiency (ANOVA $p < 0.05$) for both heparin and pentoxifylline formulations. Post hoc analysis

Table 2
Summary of *in vitro* aerosol performance (mean ± SD, $n = 3$).

	Heparin				Pentoxifylline		
	1H	1H:1L	1H:2L	2H:1L	1P:1L	1P:2L	2P:1L
Total dose (TD)[a] (mg)	96.6 ± 5.4	90.9 ± 3.3	73.6 ± 1.2	83.7 ± 3.7	39.9 ± 1.7	28.8 ± 0.5	52.1 ± 0.6
Emitted dose (ED)[b] (mg)	25.5 ± 5.0	76.1 ± 2.8	61.3 ± 2.3	71.4 ± 2.7	36.9 ± 1.9	28.3 ± 0.5	44.8 ± 3.4
Fine particle dose (FPD)[c] (mg)	12.2 ± 3.3	58.6 ± 1.6	44.1 ± 2.5	44.1 ± 2.6	9.3 ± 0.7	16.1 ± 0.7	5.2 ± 0.1
Fine particle fraction (FPF_{total})[d] (%)	12.6 ± 3.1	64.5 ± 1.3	59.9 ± 2.6	52.8 ± 4.3	22.7 ± 3.4	57.4 ± 3.1	10.1 ± 0.3
Fine particle fraction ($FPF_{emitted}$)[e] (%)	47.4 ± 3.7	77.1 ± 0.8	72.0 ± 1.4	61.7 ± 3.0	25.2 ± 3.0	57.1 ± 3.4	11.6 ± 0.9

[a] Amount of drug recovered from device, capsule and all stages of the MSLI.
[b] Amount of drug recovered from all stages of the MSLI.
[c] Amount of drug recovered with mass median aerodynamic diameter ≤5 μm.
[d] FPD/TD × 100.
[e] FPD/ED × 100.

Table 3
Effects of leucine addition on mean FPF_{TD}.

Heparin			Pentoxifylline		
Vs.		Mean difference	Vs.		Mean difference
1H	1H:1L	−51.92*	1P:1L	1P:2L	−32.74*
	1H:2L	−47.31*		2P:1L	13.3*
	2H:1L	−40.17*	1P:2L	1P:1L	32.74*
1H:1L	1H	51.92*		2P:1L	46.07*
	1H:2L	4.61	2P:1L	1P:1L	−13.33*
	2H:1L	11.75*		1P:2L	−46.07*
1H:2L	1H	47.31*			
	1H:1L	−4.61			
	2H:1L	7.14			
2H:1L	1H	40.17*			
	1H:1L	−11.75*			
	1H:2L	−7.14			

* The mean difference is significance at the 0.05 level.

(Tukey pair-wise) portrayed significant differences ($p < 0.05$; see Table 3) between FPFs of pairs of formulations except between 1H:1L vs. 1H:2L; and 1H:2L vs. 2H:1L, suggesting the quantity of leucine added to heparin formulations did not affect FPF to the same extent as for pentoxifylline. It is known that increased contact area between adjacent surfaces alters packing geometry and cohesive behaviour, which directly relates to the amount of force required to re-disperse the powder formulation (Chew et al., 2005; Adi et al., 2008c; Chew and Chan, 2001). The slight reduction in FPF in 2H:1L may be explained by differences in degrees of particle corrugation.

In contrast to heparin formulations, increasing leucine to maximum saturation at 2% (w/w) in spray-drying feed solution correlated with superior dispersion profile for pentoxifylline. 1P:2L exhibited the highest FPF_{TD} (57.4 ± 3.1%) in pentoxifylline formulations, which was substantially reduced to 10.1 ± 0.3% when the ratio was reversed to produce 2P:1L (Table 2). 1P:1L also produced a low FPF_{TD} of 22.7 ± 3.4%, which highlighted the significance of leucine contribution to improvement of aerosolisation efficiency. Post hoc statistical evaluation between different pentoxifylline formulations further supported this proportion (Table 3). Pentoxifylline particles are generally quite "adherent" with high surface energy due to its elongated needle-like shape. The anti-adherent properties of leucine thus serve an important influence on the aerosolisation of pentoxifylline formulations by reducing surface energies and inhibiting particle agglomeration. Both 1P:1L and 2P:1L deposited extensive amounts of powder on stage 1 of the MSLI with gradual reduction in amount deposited as the powder progressed through the MSLI (Fig. 5B). Of particular interest for 2P:1L, which demonstrated variable particle size distribution in laser diffraction, the slight increase in amount of powder captured by the filter membrane suggested there may be substantial amount of smaller fragment particles (below 1.7 μm) present among larger agglomerates relative to 1P:1L. Greater powder retention in the capsule by 2P:1L is also not ideal especially for dispersion using the Aeroliser® device, which relies on a capsule system. Furthermore, from the perspective of particle size analysis, both 1P:1L and 1P:2L produced similar particle size distributions with median volume diameters (D_{50}) less than 3 μm (Table 1). However, FPFs of pentoxifylline formulations significantly increased when the amount of leucine in formulation was nearly doubled from 22.7 to 57.4%, indicating particle size does not always have a direct correlation with aerosol performance.

It is important to also note that in aerosol performance analysis, only one inspiratory flow rate (60 L/min) was chosen since it is characteristic of a typical deep inhalation by a healthy adult (normal inspiration with no device). However, this may be lower in patients with smoke inhalation injury. In examining DPI devices available in the market, the mechanism of the Aeroliser® device works by powder emptying from the pierced capsule loaded into the device upon inhalation, which subsequently disperses through a grid into the mouthpiece. The narrow neck of the Aeroliser® device aids in improving potential for powder re-dispersion arising from the production of increased velocity of air currents through the channel. This efficiency of the Aeroliser® device is further enhanced by its low airflow resistance [0.06 cm H_2O/(L/min)] relative to other DPI devices such as a Turbuhaler® DPI device, for example, which has resistance of 0.1 cm H_2O/(L/min) (Chew and Chan, 2001). Using the Aeroliser® device, a healthy patient can generate around 150 L/min equivalent air flow at maximum inspiratory effort of 80 cm H_2O but at a comfortable inspiratory effort of 40 cm H_2O, a patient with compromised lung function can still generate approximately 105 L/min through the Aeroliser® (Chew and Chan, 2001). As a consequence, the Aeroliser® DPI device is likely to be the most accommodating high-efficiency device for people with impaired lung function. In a study investigating the drug delivery of eformoterol fumarate dehydrate for relief of asthma symptoms, the Aeroliser® device demonstrated an emitted dose of 90% at higher air flow rates (90 L/min) but only decreased to 80% when air flow rate was reduced to 30 L/min (Chew and Chan, 2001). Evidently, these values may not directly correspond with inhalation delivery of heparin or pentoxifylline due to differences in drug properties and formulation. Further studies exploring aerosol performance of the spray-dried powder formulations at lower air flow rates and in various dry powder devices may be useful to validate this.

Furthermore, the Aeroliser® device employs a capsule to load the dose for inhalation, which is one of its drawbacks since powder adhesion to the capsule as well as device may limit dispersion capabilities compared to a Turbuhaler® which loads a dose from a powder reservoir by a scraping mechanism for delivery using turbulent flow generated by patient inspiration (Chew and Chan, 2001). As such, the Aeroliser® device may not be suitable for use as an inhaler device to deliver drug powder formulations produced with the absence of glidant excipients such as leucine.

Preliminary studies in our research group attempted to investigate the joint pulmonary delivery of heparin and pentoxifylline by co-spray-drying drug particles with and without leucine. Despite powders conveying particle size of less than 5 μm optimal for deposition in peripheral lung areas, cascade impaction data indicated unpredictable aerosolisation characteristics. Heparin component of the heparin-pentoxifylline (HP) powder exhibited high FPF while pentoxifylline did not. This was vice versa when leucine was co-spray-dried to form heparin-pentoxifylline-leucine (HPL). The phenomenon was found to be related to non-uniform distribution of the drugs within the powders, which has particular implications on dosing consistency and limits successful delivery of sufficient amounts of both drugs to the deep lung regions where it is needed. Potential synergism effects for heparin and pentoxifylline have not been directly proven but the most analogous study by Tasaki et al. (2002) who explored nebulised heparin co-administered with an IV infusion of lisofylline (pro-drug to pentoxifylline). Co-administration in smoke-exposed sheep reduced peroxidation and enhanced blood flow to poorly ventilated areas to a greater extent than each drug alone, which translated to reduced need for mechanical ventilation and oxygen supplementation (Tasaki et al., 2002). Hence, this validates the continued need to improve joint heparin-pentoxifylline formulations, either by modification of the drug ratio used in the formulation or use of an alternative manufacture method to achieve better outcomes.

4.6. *In vitro cell toxicity of powder formulations*

No studies have investigated the cell toxicity of either inhaled heparin or pentoxifylline. One milligram per millilitre was, therefore, selected as an arbitrary standard dose to investigate for all spray-dried powder formulations. The cell viability of A549 lung

epithelial cells following 24 h exposure to the powders was, subsequently, evaluated with reference to the untreated negative control (Fig. 7). In contrast to pentoxifylline formulations, the lack of statistical differences between heparin-containing formulations and the control implied an absence of toxicity at the dose tested for heparin formulations. In clinical practice, heparin is currently administered using intravenous infusions or subcutaneous injections and to date, the equivalent dose of inhalable heparin needed to be administered to the lower respiratory tract has not been clinically established and is highly variable between studies attempting to determine equivalent inhalable doses using nebulised heparin (Bendstrup et al., 1999). Heparin dose is usually expressed in international units per mL (IU/mL) but the variations in biological activities between different preparations means that there exists no definitive standard conversion algorithm between IU and milligrams. The heparin used in this study obtained from Celsus Laboratories had an assayed biological activity of $\geq$160 IU/mg, which suggested A549 lung epithelial cells could tolerate more than $\geq$160 IU of heparin over 24 h of exposure.

On the contrary, pentoxifylline formulations engendered a statistically significant reduction in cell viability compared to the control group. The cell viability in the presence of leucine is acceptable relative to the control, which suggests the pentoxifylline component may be responsible for the relative toxicity of pentoxifylline-leucine formulations under the conditions studied. A further reduction in cell viability when the ratio of pentoxifylline was doubled from 1P:1L to 2P:1L additionally exemplified this observation. The dose of 1 mg/mL of each pentoxifylline formulation was therefore sufficient to reduce cell viability over 24 h. However, the obtained data were limited by the single dose and time conditions tested. Therefore the power of the results may be improved by conducting MTT assays over a greater spectrum of concentrations and longer exposure time points in order to establish the true toxicological profile of inhalable pentoxifylline powders. The quantity of leucine that can be tolerated by these cells could also be explored by such method. *In vivo* studies using animal models would be useful to establish the lung release profile of aerosol preparations of heparin and pentoxifylline, the drug absorption rate in lung tissue, and finally the equivalent inhaled doses required for treatment of smoke inhalation injury.

5. Conclusion

The potential use of inhalable heparin and pentoxifylline aerosol formulations as a DPI dosage form is limited by cohesive properties of the spray-dried material. The present study has demonstrated that the manufacture of inhalable heparin and pentoxifylline aerosol formulations can be successfully achieved using spray-drying as the particle engineering technique. The results indicated the co-spray-drying of heparin or pentoxifylline with leucine supplementation improved the inhalable fraction of the active material, thereby optimising its likely deposition in peripheral lung areas where it is needed for pharmacological action. It is clear the wrinkled particle morphology of leucine as observed in SEM images can reduce the magnitude of inter-particulate forces and agglomeration capability for better drug powder dispersion. With the exception of 2P:1L, particle size analysis indicating similar size distributions for all drug-leucine formulations, suggesting particle size was independent of the drug-to-leucine ratio. This sizing effect notably translated to the aerosolisation profiles of the formulations, in which all aforementioned composite powders produced satisfactory FPF_{TD} with 1H:1L and 1P:2L conveying highest fine particle fractions. Lastly, analysis of *in vitro* cell viability data using human lung epithelial cells obtained following 24 h exposure to powder formulations denoted relative toxicity of pentoxifylline-containing formulations owing to the active drug itself, which may be related to the dose and conditions investigated. Overall, this study has shown that the delivery of heparin and pentoxifylline via DPI is a feasible alternative and with further improvements to the formulation characteristics may provide an efficient manner to improve therapeutic outcomes for smoke inhalation.

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