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Characterization of nebulized buparvaquone nanosuspensions—effect of nebulization technology

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Abstract

The poorly soluble drug buparvaquone is proposed as an alternative treatment of *Pneumocystis carinii* pneumonia (PCP) lung infections. Physically stable nanosuspensions were formulated in order to deliver the drug at the site of infection using nebulization. The aerosolization characteristics of two buparvaquone nanosuspensions were determined with commercial jet and ultrasonic nebulizer devices. Aerosol droplet size distribution was determined with laser diffractometry (LD). Nebulization of the nanosuspensions and dispersion media surfactant solutions produced aerosol droplets diameters in the range from 3 to 5 μm for Respi-jet Kendall, Pari Turbo Boy system and Multisonic nebulizers and particles around 9–10 μm with Omron U1. Fractions of the nanosuspensions from the nebulizer reservoir and of aerosol produced were collected to investigate changes in the size of the drug nanocrystals influenced by the nebulization technology. Comparisons were performed measuring the drug nanocrystals with photon correlation spectroscopy (PCS) and LD of the samples. Drug particle aggregates were detected in the fractions of aerosol collected from jet nebulizers. Nebulizer technology (jet vs. ultrasonic) showed influence on the stability of the drug particle size distribution of buparvaquone nanocrystals during the nebulization time evaluated.

Keywords: Buparvaquone, nanosuspension, aerosolization, pulmonary delivery, anti infective *Pneumocystis carinii*

Introduction

Pneumocystis carinii is an atypical fungus that causes pneumonia in immunocompromised individuals (e.g. patients who had transplants, anti-cancer chemotherapy or mainly in those with advanced HIV infection). It is an extracellular organism that is found in the alveolar spaces of the lung (Wakefield 2002). Currently recommended prophylaxis therapy (initiated when patients present CD4 + cell counts below 200/mm³) involves trimethoprim-sulfamethoxazole, dapsone and aerosolized pentamidine. In patients with intolerance to these drugs, atovaquone had been used in the treatment of mild to moderate *Pneumocystis carinii* pneumonia (PCP) (Rosenberg et al. 2001) being similarly effective or at least

showing fewer adverse effects than those drugs in use (Hughes et al. 1993, 1995, El-Sadr et al. 1998, Chan et al. 1999). However, prophylaxis failure had been observed in patients with T-helper cell counts <75 or <100/mm³, a severe degree of immunosuppression that still requires a more effective regimen against PCP (Saah et al. 1995).

In previous investigations, it was found that buparvaquone exhibits more effectiveness against PCP than atovaquone, an hydroxy-1,4-naphthoquinone chemically related to buparvaquone (Kaneshiro et al. 2001). Buparvaquone (see Figure 1), is a highly lipophilic molecule with very low aqueous solubility (<1 mg/m³) that predicts a limited oral bioavailability. To improve bioavailability and to target *P. carinii*

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Figure 1. Figure 1 Structure of buparvaquone (*trans*-2-[4-*tert*-butylcyclohexylmethyl]-3-hydroxy-1,4-naphthoquinone).

at the site of action, we follow in this work the concept of pulmonary administration by nebulizing nanosuspensions.

The size reduction of buparvaquone to drug nanoparticles was achieved by high pressure homogenisation, a technology that allows the production of homogeneous nanosuspensions and enhances bio-availability by increasing the dissolution velocity and the saturation solubility of the drug (Müller et al. 1999, Müller et al. 2001). This is expected in cases where dissolution velocity is the rate-limiting step for absorption, e.g. absorption of buparvaquone by lung tissue. In comparison to microsuspensions, when nebulizing nanosuspensions, one logically increases the probability of the drug to leave the nebulizer by means of the aerosol droplets and it is theoretically expected that drug nanoparticles will be more equally distributed into water droplets (Ostrander et al. 1999, Jacobs and Müller 2002). They can reach the lung when these aerosol droplets have an appropriate aerodynamic size diameter for lung deposition (Gonda 1981, Dahlbäck 1994).

The aerosolization of the drug formulation is one of the major challenges when designing a preparation suitable for inhalation. The particle size and the speed or airflow in which they are transported, are decisive characteristics of whether particles could reach and contact the lung surface or not. A particle's depth of penetration into the respiratory system varies inversely with size. A recommended particle diameter for pulmonary deposition is between 1 and

5 μm (O' Callaghan and Barry 1997) (assuming breathing patterns from healthy adult volunteers) and therefore, the range of interest when targeting PCP.

The choice of the nebulizer system to aerosolize a nanosuspension will be one of the main factors that will determine the alveolar delivery of buparvaquone. The nebulizer needs to produce small-sized water droplets suitable for pulmonary delivery, at the same time the nebulization process should not lead to nanocrystal aggregation. It was, therefore, the aim of this work, to characterize the aerosol produced by selected jet and ultrasonic nebulizers commercially available. Laser diffractometry (LD) was used to determine the size distribution effects of primary and secondary aerosol droplets produced from two buparvaquone nanosuspensions prepared within a specific nebulization time. Although differences between nebulizers brands probably have a greater impact than differences in the formulation to nebulize (O'Riordan 2002), it is well known that the physicochemical properties of the drug formulation can also influence the droplet size (McCallion et al. 1996). Therefore, the differences in the aerosolization behaviour of two nanosuspension formulations prepared were evaluated under the same nebulization technology. The two buparvaquone nanosuspensions in study were previously optimized with appropriate surfactant mixtures of different composition to physically stabilize the drug nanocrystals (stability data unpublished). The influence of the nebulization technology on the physical stability of buparvaquone nanocrystals was evaluated in this work by monitoring the size distribution of buparvaquone nanocrystals contained in the nebulized droplets and in the fraction remaining in the nebulizer reservoir during the aerosolization time.

Materials and methods

Chemicals and reagents

Poloxamer 188 (Lutrol F68[®]) was purchased from BASF (Ludwigshafen, Germany), polyvinyl alcohol (Mowiol 4-88[®]) from Hoechst AG (Hoechst, Germany) and glycerol 85% from Aug. Hedinger GmbH (Stuttgart, Germany).

Table I. Composition of buparvaquone nanosuspension formulations A and B and aqueous surfactant reference solutions used in the study.

Formulations	Buparvaquone (% w/w)	Poloxamer 188 (% w/w)	Polyvinyl Alcohol (% w/w)	Glycerol 85% (% w/w)
Formulation A	1.0	1.0		2.5
Formulation B	1.0	0.5	0.5	2.5
Reference 1				2.5
Reference 2		0.5	0.5	2.5
Reference 3		1.0		2.5

Figure 2. Diagram of sampling during the nebulization process for determination of the size of: Nanocrystals in nebulizer Reservoir (NR-1 to NR-3), nebulized aerosol Droplets (samples D-1 to D-3), Nanocrystals in Droplets (ND-1 and ND-2). Samples of the nanocrystals in the reservoir were taken prior to nebulization (NR-1), in the middle (NR-2) and at the end of the nebulization process (NR-3). Aerosol Droplet size was determined at the beginning (0 min), in the middle (2.5 min) and at the end of the nebulization process (5.0 min). Size of the nanocrystals in the droplets ND was determined in samples collected with a beaker during the first half (ND-1) and during the second half of the nebulization process (ND-2).

Nebulization systems

Jet nebulizers: Pari Turbo Boy system composed by a Pari LC Plus Turbo nebulizer attached to a PariBoy compressor (Pari-Werk, Starnberg, Germany) and Respi-jet Kendall (Tyco Healthcare Deutschland GmbH, Germany). Ultrasonic nebulizers: Multisonic (Otto Schill GmbH & Co. KG, Probstzella, Germany) and Omron U1 (Omron Healthcare Europe B.V., Hoofddorp, The Netherlands).

Preparation of nanosuspensions

Surfactants were dissolved in bidistilled water before use. Poloxamer 188 was dissolved at room temperature and polyvinyl alcohol was dissolved in warm water ($\sim 85^{\circ}\text{C}$). Nanosuspensions were prepared with 1% of drug using surfactant mixtures as listed in Table I (formulations A and B).

Drug powder was dispersed in each case in the aqueous surfactant solution using an Ultra-turrax T 25 (Janke und Kunkel GmbH, Staufen, Germany) for 1 min at 8000 rpm. The 50% volume diameter of drug particles in this dispersion (d50percentage of volume of the particles that are below the given size) was $86.5 \pm 9.0 \mu\text{m}$ and d99% was $465.1 \pm 24.4 \mu\text{m}$. The dispersion was homogenized using an APV Gaulin Micron LAB 40 homogenizer discontinuous version (APV Systems, Unna, Germany). Each formulation was homogenized applying two cycles at 150 bar and two at 500 bar as premilling of the product. Then, 40 homogenization cycles at 1500 bar

were run to obtain the final product. The pH, osmolarity, viscosity and density of the nanosuspensions were measured after preparation, using a pH-Meter CG840 (Schott-Geräte GmbH, Hofheim, Germany), a cryoscopic osmometer Osmomat 030 (Gonotec GmbH, Berlin Germany), rotation Ubbelohde-type viscometer AVS 350 (Schott-Geräte GmbH, Mainz, Germany) and a density meter DMA 38 (Anton Paar GmbH, Graz, Austria), respectively.

Characterization of aerosol droplet size and physical stability of the drug nanocrystals following nebulization

Five millilitres of each nanosuspension were nebulized during 5 min per nebulizer (Figure 2). An overview of the samples drawn in Figure 2 is given as follows:

- NR = Nanocrystals in the reservoir prior to nebulization (NR-1), in the middle (NR-2) and at the end of the nebulization process (NR-3).
- D = Aerosol droplet size measured at the beginning (D-1), in the middle (D-2) and at the end (D-3) of the nebulization process.
- ND = Size of nanocrystals in the droplets collected with a beaker during the first half of the nebulization process (ND-1) and during the second half of the 5 min process (ND-2)

Before starting each nebulization, a sample from the nanosuspension in the nebulizer reservoir was taken to measure drug nanocrystals size (NR-1).

Immediately after starting nebulization, VMD X_{50} (volume median diameter) of the aerosol droplets was measured ($n = 5$ for all measurements) using a HELOS laser diffractometer with sprayer module (Sympatec, Clausthal-Zellerfeld, Germany). Measurements were performed applying the method previously described by Steckel and Eskandar developed for this specific instrument, using a 20 mm lens (measuring range from 0.18 to 35 μm) and a distance of 63 mm between the nebulizer and the focus of the lens (Steckel and Eskandar 2003). The ambient conditions during the measurements were temperature $25 \pm 1^\circ\text{C}$ and a relative humidity of $50 \pm 5\%$. Standard conditions were kept throughout the experiments in order to ensure a constant influence of these parameters in the measurements regarding evaporation and condensation of solvent water on the surface of the droplets. During the measurements of aerosol droplet size, a suction pump was connected to remove the aerosol from the atmosphere and to avoid re-entering of the particles to the path of the laser beam. Immediately after measuring the droplet size of the aerosol (D-1), the vacuum system was replaced by a glass beaker, whereby aerosol droplets were collected for later measurement of the drug nanocrystals size (ND-1). After 2.5 min the nebulization was stopped, and a second sample was taken from the nebulizer reservoir to measure the size of nanocrystals (NR-2). Nebulization was immediately restarted and aerosol droplets were again measured with LD (D-2). Again aerosol droplets were collected with a glass beaker for nanocrystals size measurement (ND-2); aerosol droplets were once again measured at the end of the nebulization process (D-3) and a sample of residual nanosuspension was taken from the nebulizer reservoir (NR-3). All nanosuspension fractions collected from the reservoir (NR) and from the suspension in the droplets (ND) were analysed for particle size characterization and determination of possible aggregation of the buparvaquone drug nanocrystals.

References of the dispersion media used in the preparation of the nanosuspensions prepared as listed in Table I (reference solutions 1–3) were also tested for aerosol droplet size distribution under the same conditions described above. The parameters considered to characterize the aerosol droplets (D-1 to D-3) produced by the four selected nebulizers with the two buparvaquone formulations and the reference solutions in study, included the mass median diameter (MMD) calculated from the VMD X_{50} assuming spherical unit density particles, the percentage of aerosol droplets under 5 μm , to evaluate the potential quantity of droplets that can actually reach the lungs, and the span value which was calculated using the known formula

by McCallion et al. (1995):

$$\text{Span} = \frac{(90\% \text{ undersize} - 10\% \text{ undersize})}{50\% \text{ undersize}}$$

Comparisons of the aerosol droplet size distribution values (MMD and span) between nanosuspension formulations A and B (and each formulation against their respective dispersion media, references 1–3) are performed for each nebulizer device using a *t*-test with a level of significance of 5%.

Particle size characterization of nanocrystals from the reservoir (NR) and nanocrystals in the droplets (ND)

Laser diffraction (LD) analysis of the samples NR to ND were performed using the HELOS Laser diffractometer (Sympatec, Clausthal-Zellerfeld, Germany) installing the 6 ml cuvette module. During the analysis, samples were diluted with distilled water to achieve the appropriate concentration for the analysis. The particle diameters were calculated by using the volume distribution. Diameters d50, d90 and d99% were investigated (percentage of volume of the particles that are below the given size). Particle size characterization with Photon Correlation Spectroscopy (PCS) was done using a Malvern Zetasizer 4 (Malvern Instruments Ltd., Malvern, UK). Samples were also diluted for this analysis with distilled water. All samples were analysed for LD and PCS ($n = 3$).

Results and discussion

Nanoparticle preparation and characterization

Nanosuspensions were prepared considering their use for pulmonary delivery. Their osmolality was adjusted to be within the physiological range using glycerol 85% in the formulations and the pH value of 7.0 obtained for the final preparation was considered appropriate. The particle size distribution of the drug nanocrystals as well as the summary of the physical

Table II. Summary of the nanocrystal characteristics (mean $\pm$ SD; $n = 3$) measured with PCS and LD before the nebulization process and physical properties of the nanosuspension.

Parameter	Formulation A	Formulation B
PCS average diameter (nm)	473 $\pm$ 15	406 $\pm$ 8
PCS Polydispersity Index	0.34 $\pm$ 0.01	0.25 $\pm$ 0.05
d50% (μm)	0.9 $\pm$ 0.0	0.8 $\pm$ 0.0
d90% (μm)	1.6 $\pm$ 0.1	1.5 $\pm$ 0.1
d99% (μm)	2.6 $\pm$ 0.3	2.5 $\pm$ 0.3
PH	7.0 $\pm$ 0.05	7.0 $\pm$ 0.05
Osmolality (osmol/kg)	0.333 $\pm$ 0.003	0.336 $\pm$ 0.002
Viscosity (mPa s)	1.2 $\pm$ 0.02	1.3 $\pm$ 0.01
Density (g/cm ³)	1.005 $\pm$ 0.00	1.006 $\pm$ 0.00

properties (osmolarity, density and viscosity) of the nanosuspensions before the nebulization process is listed in Table II. The quality of both formulations is acceptable for the intended use; the LD diameter d99% was around 2.5 μm for both formulations, which predicts that most of the nanocrystals have the appropriate particle size distribution to be incorporated into the aerosol droplets of adequate size to reach the lungs. Formulation B showed a less polydisperse distribution with drug particles of smaller average diameter.

Characterization of the droplets in the nebulized aerosol (D-1 to D-3)

The mass median diameter (MMD) is described as the diameter of an aerosol particle such that, half of the mass of the aerosol is contained in smaller diameter particles and half in larger. While this parameter represents the average of the droplet size volume distribution, the parameter span represents the width of this distribution relative to the median diameter. These values, as well as the percentage of particles emitted within the respirable range ($\% < 5 \mu\text{m}$) produced by the selected nebulizers, are showed in Tables III and IV. These data describe the aerosolization droplets produced with the two buparvaquone nanosuspensions prepared, including the information obtained when nebulizing the surfactant solutions, as references of aerosol droplet size without drug nanoparticles.

The influence of viscosity on the nebulization process of jet and ultrasonic nebulizers has been reported before (McCallion et al. 1995). The viscosity of the reference surfactant solutions was measured. The reference 1, a solution prepared without surfactants, showed a viscosity of 1.04 mPa s, while references 2 and 3 (see Table I for composition) showed viscosity values of 1.31 and 1.20 mPa s, respectively. When nebulizing these solutions with ultrasonic nebulizers to determine the size of the droplets produced, higher diameter values were obtained from references 2 and 3 compared to reference 1 with Omron U1 nebulizer. These observations were consistent with the literature, where an increment in MMD proportional to the viscosity was also observed with this technology. However, differences in viscosity within the range of the references evaluated, showed a negligible effect with other devices (Multisonic, Respi-jet Kendall or Pari Turbo Boy) on the droplet size distribution measured within 5 min nebulization. Nanosuspension formulations A and B presented similar behaviour as that observed for the reference solution and their viscosity was not different from their respective reference solution 2 and 3 (see values in Table II).

The two jet nebulizers selected have the same mechanism of action. In these systems, the aerosol is

generated by a high speed air flow shearing the liquid into small droplets (primary generation). The generation process is improved by impaction of the droplets in a baffle structure transferring kinetic energy further into increased droplet surface area (secondary generation). The resulting droplet size distribution still contains only a small fraction of droplets below 5 μm in size, and the large droplets are recirculated within the nebulizer by means of impaction structures (Knoch and Sommer 2000). In this work, the dynamic flow measured for both Pari Turbo Boy and Respi-jet Kendall was around 6 l/min. The high operation flow of both nebulizers as well as their baffles design, allow us to obtain droplets in the range of 4–4.5 μm for both nanosuspensions without showing any tendency in the size within the 5 min tested. Analyzing the data presented in Table III, and considering a 5% level of significance when performing the t-test comparison of average aerosol droplet sizes, no significant difference was observed when nebulizing nanosuspension A or B with any of both nebulizers.

When nebulizing each of the nanosuspensions (A or B) with the ultrasonic nebulizers selected, notable differences are observed (see Table IV) dependent on the device used and not on the physical properties and composition of the formulation. Multisonic nebulizer produced droplets of around 4.7 μm with both formulations, while the Omron U1 nebulizer produced aerosol particles of around 9.5 μm , droplets too large to penetrate the lower respiratory tract. The aerosolization behaviour was expected to be different considering the properties of each nebulizer. The Omron U1 nebulizer is mesh technology based. It uses the vibration of the piezoelectric crystal to generate an aerosol indirectly. It is a battery-operated hand-held nebulizer, that produces droplets when the crystals vibrate around a feeding tube turning it into a peristaltic pump. It forces liquid through a ceramic mesh (pore size 4.6 μm) creating the aerosol cloud at a low flow rate. The frequency of operation of this nebulizer is 65 kHz, which results in a low nebulization rate of 0.25 ml/min. Well known limitations of this device include an inability to control nebulization of drugs present as a micronized suspension (e.g. glucocorticoids) due to incorporation of a small mesh hole size (4.6 μm) which could trap suspension particles (Dennis and Nierbrink 2002). Although, the use of drug nanocrystals in the average size produced in this work ($\sim 400 \text{ nm}$) was an option to overcome that situation, the high particle size of the droplets produced makes it unattractive for further investigations.

The Multisonic nebulizer has a different principle of operation. Ultrasonic vibrations from the crystal are transmitted to the surface of the drug solution where standing waves are formed. Droplets break free from the crests of these waves and are released as aerosol. The size of droplets produced is inversely proportional

Table III. LD diameters of aerosol droplets (mean $\pm$ SD; $n = 5$) produced during jet nebulization of buparvaquone nanosuspension (Formulations A and B) and references (1,2 and 3). Measurements D-1, D-2 and D-3 correspond to time 0, 2.5 and 5 min nebulization time (see diagram Figure 2).

	Respi-jet Kendall			Pari Turbo Boy		
	MMD*	Span	% < 5 μ m	MMD	Span	% < 5 μ m
<i>Formulation A</i>						
Measurement D-1	4.1 $\pm$ 0.0	2.0 $\pm$ 0.0	60.0 $\pm$ 0.6	4.3 $\pm$ 0.1	2.5 $\pm$ 0.1	56.0 $\pm$ 1.0
Measurement D-2	4.0 $\pm$ 0.0	1.9 $\pm$ 0.0	63.7 $\pm$ 4.5	4.1 $\pm$ 0.2	2.7 $\pm$ 0.1	57.9 $\pm$ 1.8
Measurement D-3	4.4 $\pm$ 0.0	1.6 $\pm$ 0.0	59.2 $\pm$ 0.8	4.2 $\pm$ 0.1	2.6 $\pm$ 0.1	57.1 $\pm$ 1.1
<i>Formulation B</i>						
Measurement D-1	4.5 $\pm$ 0.1	1.9 $\pm$ 0.0	54.7 $\pm$ 0.5	4.6 $\pm$ 1.0	2.4 $\pm$ 0.6	50.3 $\pm$ 2.6
Measurement D-2	4.4 $\pm$ 0.1	2.0 $\pm$ 0.0	60.7 $\pm$ 1.4	4.3 $\pm$ 0.0	2.6 $\pm$ 0.0	56.1 $\pm$ 0.3
Measurement D-3	4.6 $\pm$ 0.1	2.0 $\pm$ 0.1	55.7 $\pm$ 1.1	4.1 $\pm$ 0.1	2.7 $\pm$ 0.1	57.7 $\pm$ 1.0
<i>Reference 1</i>						
Measurement D-1	4.4 $\pm$ 0.1	2.0 $\pm$ 0.0	60.4 $\pm$ 2.3	4.2 $\pm$ 0.5	2.4 $\pm$ 0.4	54.0 $\pm$ 1.0
Measurement D-2	4.3 $\pm$ 0.2	0.9 $\pm$ 0.6	71.5 $\pm$ 6.0	3.8 $\pm$ 0.5	2.6 $\pm$ 0.2	58.2 $\pm$ 1.4
Measurement D-3	4.4 $\pm$ 0.2	2.1 $\pm$ 0.0	62.3 $\pm$ 2.1	3.4 $\pm$ 0.5	2.6 $\pm$ 0.2	62.0 $\pm$ 1.0
<i>Reference 2</i>						
Measurement D-1	4.7 $\pm$ 0.0	1.9 $\pm$ 0.0	53.4 $\pm$ 0.3	4.6 $\pm$ 0.2	2.3 $\pm$ 0.1	53.3 $\pm$ 1.6
Measurement D-2	4.6 $\pm$ 0.1	1.9 $\pm$ 0.0	53.8 $\pm$ 0.9	4.7 $\pm$ 0.2	2.3 $\pm$ 0.1	51.9 $\pm$ 1.5
Measurement D-3	4.2 $\pm$ 0.1	2.1 $\pm$ 0.0	58.1 $\pm$ 0.9	4.5 $\pm$ 0.2	2.3 $\pm$ 0.1	53.2 $\pm$ 1.1
<i>Reference 3</i>						
Measurement D-1	4.6 $\pm$ 0.1	1.8 $\pm$ 0.0	54.1 $\pm$ 0.6	3.9 $\pm$ 0.1	2.7 $\pm$ 0.0	60.4 $\pm$ 0.7
Measurement D-2	4.3 $\pm$ 0.2	1.6 $\pm$ 0.1	58.4 $\pm$ 3.4	3.8 $\pm$ 0.2	2.8 $\pm$ 0.1	59.3 $\pm$ 1.2
Measurement D-3	3.9 $\pm$ 0.1	1.7 $\pm$ 0.1	63.2 $\pm$ 2.6	3.7 $\pm$ 0.1	2.9 $\pm$ 0.0	61.8 $\pm$ 1.3

* Original VMD (volume median diameter) measurements correlated to MMD (mass median diameter) assuming the density of nebulized droplets equals to unity.

to two thirds of the power of the acoustic frequency (O'Callaghan and Barry 1997). Like jet nebulizers, baffles within the nebulizer remove large droplets and much of the aerosol produced impacts on these, falling

back into the drug reservoir. The nebulization frequency of operation is 1.7 MHz, an advantage that explains a higher nebulization rate of 0.75 ml/min and higher efficiency to produce droplets

Table IV. LD diameters of aerosol droplets (mean $\pm$ SD; $n = 5$) produced during ultrasonic nebulization of buparvaquone nanosuspension (Formulations A and B) and references (1,2 and 3). Measurements D-1, D-2 and D-3 correspond to time 0, 2.5 and 5 min nebulization time (see diagram Figure 2).

	Multisonic			Omron U1		
	MMD*	Span	% < 5 μ m	MMD	Span	% < 5 μ m
<i>Formulation A</i>						
Measurement D-1	4.5 $\pm$ 0.1	1.4 $\pm$ 0.1	57.7 $\pm$ 1.8	9.5 $\pm$ 1.1	1.3 $\pm$ 0.0	27.4 $\pm$ 5.7
Measurement D-2	4.7 $\pm$ 0.3	1.3 $\pm$ 0.1	54.5 $\pm$ 4.4	9.2 $\pm$ 0.2	1.3 $\pm$ 0.1	21.8 $\pm$ 2.3
Measurement D-3	5.0 $\pm$ 0.1	1.6 $\pm$ 0.1	49.6 $\pm$ 1.3	10.0 $\pm$ 0.1	1.3 $\pm$ 0.0	17.8 $\pm$ 0.6
<i>Formulation B</i>						
Measurement D-1	4.6 $\pm$ 0.2	1.3 $\pm$ 0.1	58.4 $\pm$ 5.9	9.5 $\pm$ 0.2	1.3 $\pm$ 0.1	20.1 $\pm$ 1.1
Measurement D-2	5.0 $\pm$ 0.3	1.6 $\pm$ 0.1	51.2 $\pm$ 5.0	9.6 $\pm$ 0.4	1.3 $\pm$ 0.0	20.0 $\pm$ 1.2
Measurement D-3	4.8 $\pm$ 0.3	1.4 $\pm$ 0.1	53.5 $\pm$ 4.6	9.3 $\pm$ 0.5	1.4 $\pm$ 0.1	17.7 $\pm$ 1.9
<i>Reference 1</i>						
Measurement D-1	5.2 $\pm$ 0.1	1.4 $\pm$ 0.1	47.7 $\pm$ 1.2	8.9 $\pm$ 0.1	1.1 $\pm$ 0.0	24.1 $\pm$ 1.6
Measurement D-2	4.6 $\pm$ 0.3	1.4 $\pm$ 0.1	56.6 $\pm$ 4.6	9.4 $\pm$ 0.6	1.1 $\pm$ 0.0	21.9 $\pm$ 1.3
Measurement D-3	4.7 $\pm$ 0.1	1.5 $\pm$ 0.0	53.8 $\pm$ 1.4	8.9 $\pm$ 0.2	1.1 $\pm$ 0.0	24.9 $\pm$ 1.3
<i>Reference 2</i>						
Measurement D-1	5.5 $\pm$ 0.3	1.7 $\pm$ 0.1	44.2 $\pm$ 3.1	10.9 $\pm$ 0.2	1.2 $\pm$ 0.0	17.5 $\pm$ 0.8
Measurement D-2	4.9 $\pm$ 0.1	1.5 $\pm$ 0.0	51.9 $\pm$ 1.6	10.6 $\pm$ 0.2	1.2 $\pm$ 0.0	19.3 $\pm$ 0.7
Measurement D-3	5.0 $\pm$ 0.2	1.6 $\pm$ 0.2	50.9 $\pm$ 3.2	10.7 $\pm$ 0.3	1.2 $\pm$ 0.0	18.5 $\pm$ 2.3
<i>Reference 3</i>						
Measurement D-1	5.5 $\pm$ 0.4	1.7 $\pm$ 0.2	44.4 $\pm$ 4.1	10.4 $\pm$ 0.4	1.2 $\pm$ 0.1	21.3 $\pm$ 1.9
Measurement D-2	4.9 $\pm$ 0.2	1.5 $\pm$ 0.1	51.0 $\pm$ 2.2	10.6 $\pm$ 0.3	1.2 $\pm$ 0.0	20.8 $\pm$ 2.2
Measurement D-3	4.7 $\pm$ 0.2	1.4 $\pm$ 0.1	55.7 $\pm$ 3.5	10.6 $\pm$ 0.4	1.2 $\pm$ 0.0	20.7 $\pm$ 1.2

* Original VMD (volume median diameter) measurements correlated to MMD (mass median diameter) assuming the density of nebulized droplets equals to unity.

Figure 3. PCS data of nanosuspension A comparing the average diameter (bars) and polydispersity index of the drug nanocrystals in the reservoir NR-1 to NR-3 (left) with the size of the nanocrystals in the aerosol droplets ND-1 and ND-2 (right) collected at various times during the 5 min nebulization.

$< 5 \mu\text{m}$. The size of the droplets produced with this technology are small enough to reach the respiratory tract. The ability of the buparvaquone nanocrystals to rise up on the crests of the waves produced requires further investigation calculating their emitted dose, before a final comparison between different devices can be made, which was not one of the aims of this work.

The dispersity of the particle size distribution (span) was smaller for the droplets produced with ultrasonic nebulizers than those produced with jet nebulizers with both formulations. Multisonic nebulizer showed a span value between 1.3 and 1.6 for both formulations, while the droplets produced by jet nebulizers showed values between 2.0 and 2.7.

The respirable percentage defined as the proportion of particles with appropriate size to reach the lung (percentage of particles $< 5 \mu\text{m}$), was a similar value for three of the nebulizers ($\sim$ between 50 and 60%): Respi-jet Kendall, Pari Turbo Boy and Multisonic with both formulations tested. The percentage of particles under $5 \mu\text{m}$ produced with Omron U1 nebulizer was considerable smaller than that obtained with any of the other devices ($\sim 20\%$), a situation

already expected due to the MMD value measured with this device and by the reference values given by the manufacturer.

Influence of nebulization on nanocrystal characteristics

The effect of the selected nebulizer technologies on the PCS particle size distribution (average size and polydispersity index) for both buparvaquone nanosuspensions tested is shown in Figures 3 and 4. Samples of nanosuspension from the nebulizer reservoir (NR, left) and nanocrystal size in the aerosol droplets collected (ND, right) were compared for each device used. According to this data, the original average particle size diameter of the drug nanocrystals remained the same for most of the fractions analyzed. Only the PCS data obtained with the Multisonic nebulizer when nebulizing nanosuspension formulation B showed a diminution from about ~ 400 to ~ 340 nm (see Table I for initial data). This difference was attributed to the fundamental technology, which the Multisonic nebulizer uses for the generation of droplets. The standing waves produced on the surface of the nebulizer nanosuspension by the vibration of a

Figure 4. PCS data of nanosuspension B comparing the average diameter (bars) and polydispersity index of the drug nanocrystals in the reservoir NR-1 to NR-3 (left) with the size of the nanocrystals in the aerosol droplets ND-1 and ND-2 (right) collected at various times during the 5 min nebulization.

Figure 5. Laser diffractometry diameters (LD50, LD90, LD99) of the buparvaquone nanocrystals in the reservoir NR-1 to NR-3 (top) and the drug nanocrystals in the aerosol droplets ND-1 and ND-2 (bottom) collected at various times during the 5 min nebulization of nanosuspension A.

piezoelectric crystal led to the formation of water droplets. Therefore, when a polydisperse nanosuspension is aerosolized, the smallest particles located on the surface of the sample will therefore be preferentially loaded into the droplets formed.

LD data of the particle size distribution of drug nanoparticles in formulations A and B following their nebulization with the selected nebulizers are represented in Figures 5 and 6. Evaluating the changes in the aerosol droplet size distribution as a function of the nebulizer device for formulation A (Figure 5), the

use of the Respi-jet Kendall showed a high aggregation of the drug nanocrystals in the collected aerosol droplets ND-1 and ND-2 (d90% $\sim 16 \mu\text{m}$ d99% $\sim 20 \mu\text{m}$). Aggregation was observed as well after the nebulization with Pari LC turbo system and Omron U1, but the aggregation was negligible and noticeable only when evaluating the d99% values (values around 4 and $3 \mu\text{m}$ for Omron U1 and Pari Turbo Boy, respectively). Other values remained without change.

For formulation B (Figure 6), aggregation was clearly observed with both jet nebulizer tested, the largest aggregates were observed by nebulization with the Respi-jet Kendall nebulizer. The characteristics of drug nanocrystals following nebulization with the Multisonic nebulizer followed the same profile observed before with PCS data. Also nebulization with Omron U1 showed no influence on the drug particle size. The aggregation phenomena observed as an increase in laser diameter of the samples (ND-1, ND-2) can be explained by the water evaporation effect known during the process, occurring when the jet high velocity gas is passed through a narrow venturi nozzle and facilitating the aggregation of particles leaving the nebulizer device.

Conclusions

The present work shows that the drug buparvaquone can be successfully formulated as nanosuspension by high pressure homogenization, yielding drug nanocrystals with acceptable diameter to be incorporated into water aerosol droplets and—from the measured droplet size—can reach the site of action in the lungs. The buparvaquone nanosuspension formulations A and B can be nebulized with jet and ultrasonic technologies. From the nebulizers tested, Omron U1 produced droplets over the desirable range for both formulations tested, also when measuring droplets produced from reference surfactant solutions.

Figure 6. Laser diffractometry diameters (LD50, LD90, LD99) of the buparvaquone nanocrystals in the reservoir NR-1 to NR-3 (left) and the drug nanocrystals in the aerosol droplets ND-1 and ND-2 (right) collected at various times during the 5 min nebulization of nanosuspension B.

P. carinii therapy or prophylaxis via inhalation requires penetration of the drug to the peripheral lung region and the appropriate droplet size distribution of the aerosol to be inhaled will be determinant for their deposition. The aerosolization of both formulations produced droplets in the range 3–5 µm using any of the jet nebulizers tested (Pari Turbo Boy and Respi-jet Kendall) and the piezoelectric ultrasonic nebulizer (Multisonic), an appropriate value to predict central and peripheral deposition. During the nebulization process, the three samples of the aerosol droplet (D-1–D-3) showed similar size values.

However, an influence on the size distribution characteristics of the buparvaquone nanocrystals in the aerosol droplets was observed following nebulization. Increment in laser diameters measured indicated pronounced aggregation especially in fractions collected from the jet nebulizer Respi-jet Kendall for formulation A. The Pari Turbo Boy system showing little effect on nanocrystal size with formulation A is also considered a suitable device. In formulation B, some aggregation was confirming that the quality of the nebulized product depends on device and formulation composition (i.e. type of stabilizer). The ultrasonic nebulizer Multisonic proved to be the most suitable to aerosolize the drug nanosuspensions, without any formulation dependency (at least for the investigated formulations), i.e. in terms of physical nanocrystal stability in the reservoir and nanocrystal stability in the aerosol droplets, producing simultaneously an acceptable droplet size for pulmonary deposition.

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References

- Chan C, Montaner J, Lefebvre E-A, Morey G, Dohn M, McIvor RA, Scott J, Marina R, Caldwell P. 1999. Atovaquone suspension compared with aerosolized pentamidine for prevention of *Pneumocystis carinii* pneumonia in human immunodeficiency virus-infected subjects intolerant of trimethoprim or sulfonamides. *J Infect Dis* 180:169–176.
- Dahlbäck M. 1994. Behavior of nebulizing solutions and suspensions. *J Aerosol Med* 7:S13–S18.
- Dennis JH, Nierbrink O. 2002. New nebulizer technology. In: Bisgaard H, O'Callaghan CO, Smaldone GC, editors. *Drug delivery to the lung*. New York: Marcel Dekker. p 303–336.
- El-Sadr WM, Murphy RL, McCabe Yurik T, Luskin-Hawk R, Cheung TW, Balfour HH, Eng R, Hooton TM, Kerkering TM, Schutz M, et al. 1998. Atovaquone compared with Dapsone for the prevention of *Pneumocystis carinii* pneumonia in patients with HIV infection who cannot tolerate trimethoprim, sulfonamides, or both. *New Eng J Med* 339:1889–1995.
- Gonda I. 1981. A semi-empirical model of aerosol deposition in the human respiratory tract for mouth inhalation. *J Pharm Pharmacol* 33(11):692–696.
- Hughes W, Leoung G, Kramer F, Bozzette SA, Safrin S, Frame P, Clumeck N, Masur H, Lancaster D, Chan C, et al. 1993. Comparison of Atovaquone (566C80) with trimethoprim-sulfamethoxazole to treat *Pneumocystis Carinii* pneumonia in patients with AIDS. *New Eng J Med* 328:1521–1527.
- Hughes WT, LaFon SW, Scott JD, Masur H. 1995. Adverse events associated with trimethoprim-sulfamethoxazole and atovaquone during the treatment of AIDS-related *Pneumocystis carinii* pneumonia. *J Infect Dis* 171:1295–1301.
- Jacobs C, Müller RH. 2002. Production and characterization of a budesonide nanosuspension for pulmonary administration. *Pharm Res* 19:189–194.
- Kaneshiro ES, Sul D, Basselin M, Kayser O. 2001. *Pneumocystis carinii* synthesizes four ubiquinone homologs: Inhibition by atovaquone and buparvaquone but not by stigmatellin. *J Eukaryot Microbiol (Suppl)*:1725–1733.
- Knoch M, Sommer E. 2000. Jet nebulizer design and function. *Eur Respir Rev* 10:183–186.
- McCallion ONM, Taylor KMG, Thomas M, Taylor AJ. 1995. Nebulization of fluids of different physicochemical properties with air-jet and ultrasonic nebulizers. *Pharm Res* 12:1682–1688.
- McCallion ONM, Taylor KMG, Bridges PA, Thomas M, Taylor AJ. 1996. Jet nebulisers for pulmonary drug delivery. *Int J Pharm* 130:1–11.
- Müller RH, Becker R, Kruss B, Peters K. 1999. Pharmaceutical nanosuspensions for medicament administration as system of increased saturation solubility and rate of solution. *US patent* 5,858,410.
- Müller RH, Jacobs C, Kayser O. 2001. Nanosuspensions as particulate drug formulations in therapy rationale for development and what we can expect for the future. *Adv Drug Deliv Rev* 47:3–19.
- O'Riordan TG. 2002. Formulations and nebulizer performance. *Respir Care* 47:1305–1312.
- O' Callaghan C, Barry PW. 1997. The science of nebulised drug delivery. *Thorax* 52:S31–S44.
- Ostrander KD, Bosch WH, Bondanza DM. 1999. An *in-vitro* assessment of a nanocrystal (beclomethasone dipropionate colloidal dispersion via ultrasonic nebulization. *Eur J Pharm Biopharm* 48:207–215.
- Rosenberg DM, McCarthy W, Slavinsky J, Chan CKN, Montaner J, Braun J, Dohn MN, Caldwell PT. 2001. Atovaquone suspension for treatment of *Pneumocystis carinii* pneumonia in HIV-infected patients. *AIDS* 15:211–214.
- Saah AJ, Hoover DR, Peng Y, Phair JP, Visscher B, Kingsley LA, Schrager LK. 1995. Predictors for failure of *Pneumocystis carinii* pneumonia prophylaxis. Multicenter AIDS Cohort Study. *JAMA* 273:1197–1202.
- Steckel H, Eskandar F. 2003. Factors affecting aerosol performance during nebulization with jet and ultrasonic nebulizers. *Eur J Pharm Sci* 19:443–455.
- Wakefield AE. 2002. *Pneumocystis carinii*. *Br Med Bull* 61:175–188.