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Evaluation of the delivery of suspensions from an ultrasonic nebuliser with and without the lung surfactant 'Exosurf'

U.T. Lashmar [a], D. Provasi [b] and S.W. Poole [a]

[a] *Pharmaceutical Development Laboratories, The Wellcome Foundation Plc, Dartford (UK) and* [b] *Department of Pharmacy, Parma University, Parma (Italy)*

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Summary

A multistage liquid impinger (MLI) was used to determine the fraction of spherical particles delivered within the range of 5–0.3 μm, from 0.25% w/w suspensions of five different sizes of spheres alone or together with the lung surfactant 'Exosurf' by means of an ultrasonic nebuliser, the fraction of Exosurf delivered was also determined. Only a small proportion of the spheres was delivered to the MLI from suspensions of the spheres. The largest amount collected was 18% w/w of the loaded amount and this was obtained from a suspension of spheres with a nominal size of 0.35 μm. This was in part found to be due to the presence of aggregates of the spheres in the nebulising chamber, which formed during sonication of the suspension. The addition of Exosurf to the suspensions increased the total amount of spheres impacted in the MLI for all the sphere sizes tested. High concentrations of Exosurf increased the proportion of spheres delivered to the MLI most, and large spheres benefitted most from the addition of Exosurf. These results suggested that the formation of aggregates by the nebuliser was reduced when Exosurf was added to the suspension. The amount of Exosurf and spheres delivered to the MLI was also found to have increased when mixtures of spheres and Exosurf were nebulised, possibly due to interactions between the two.

Introduction

Coadministration of a lung surfactant with a drug for pulmonary administration has been shown to extend the area of administration within the lung, alter the pharmacokinetics of a drug and improve the localised drug action within the respiratory tract (Juliano and McCullough, 1980; Gilbert et al., 1988; Taylor et al., 1989; Kharasch et al., 1991; Barker, 1992). The most convenient way of administering drugs to the lungs is by means of inhalation. It has been shown that nebulisers only deposit a small fraction of their dose in the lung (Byron, 1986) and that nebuliser efficiency is dependent on the brand of nebuliser used (Smith et al., 1992). The purpose of this study was to determine the effect of coadministration of a lung surfactant on the amount of drug of various particle sizes reaching the lungs in vitro when administered by means of an ultrasonic nebuliser. The lung surfactant Exosurf was

Correspondence to: U.T. Lashmar, Pharmaceutical Development Laboratories, The Wellcome Foundation Plc, Dartford, U.K.

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used since it has been developed for the treatment of respiratory distress syndrome and, therefore, is accepted for pulmonary delivery, it is also known to have a good lung distribution (Harwood et al., 1992). The literature (Bell et al., 1973; Smith et al., 1992) describes a variety of instruments designed for aerosol sizing and instruments designed to mimic the lungs. This present study used a calibrated multistage liquid impinger (MLI) since the MLI is particularly useful for in vitro aerosol characterisation, as the presence of water at the impaction site resembles the humid conditions of the human lung.

Materials and Methods

Materials

Fluoresbrite polybeads with a nominal size of 0.35, 0.75, 1.0, 2.0 and 3.0 μm were obtained from Polysciences as 2.5% w/v suspensions. Actual sizes were determined by a Coulter multisizer II and Coulter sub-micron particle analyser model N4MD. Exosurf Paediatric Sterile Powder (Wellcome, U.K.) lot no. 56813 was used. Exosurf contains 63% w/w dipalmitoylphosphatidylcholine together with other surfactants. Methanol 205 and Acetonitrile 190 were obtained from Romil Chemicals and choline chloride from Sigma.

Formulations

Each tested nebuliser suspension contained 0.25% w/v fluoresbrite spheres with a nominal diameter of 3.0, 2.0, 1.0, 0.75 or 0.35 μm and 0.22, 0.044, 0.022 or 0.0% w/v Exosurf or 2.2, 0.22 or 0.022% w/v Exosurf alone. The suspensions were made up to volume with deionised water.

Nebuliser

A Penta-Sonic Ultrasonic Nebuliser (Devilbiss) filled with 2–5 ml suspension was used for the study. The stated nebuliser output was 0.5 ml per min.

Characterisation of aerosols

Aerosols generated from the ultrasonic nebuliser were characterised using a wet stage cascade impactator called a multistage liquid impinger (MLI). The MLI first described by May (1966) was used. Methanol or water was placed in each of the upper stages until the level just reached the lower surface of the collection plate, the final stage being filled with 50 ml methanol or water.

Calibration of the MLI

The MLI was calibrated with respect to the effective cut off diameter (ECD) for each stage, at a flow rate at 60 l/min. The flow rate used in the study was 20 l/min and the ECD values were, therefore, adjusted as described by May (1966). The ECD, which is the 50% cut off diameter for the particles, was determined for each stage by the Atomic Energy Establishment employing a spinning disc generator. For information about the procedure see Camner et al. (1971). The ECD of the throat was 40 μm; stage 1, 10.0 μm; stage 2, 4.6 μm and stage 3, 2.0 μm.

Analysis of fluoresbrite polybeads

The fluoresbrite polybeads were assayed by UV at 292 nm using a Perkin-Elmer 555 and employing an $E^{1\%}$ determined for each of the sphere sizes.

Analysis of Exosurf

Exosurf was assayed with respect to dipalmitoylphosphatidylcholine (DPPC) content by means of HPLC using a Constametric 3000 pump (LDC) an autoinjector (Shimadzu), a variable wavelength detector spectromonitor 3100 (LDC) and a CI 4100 integrator (LDC). The wavelength of the detector was set at 214 nm. The column was a Whatman Partisil 5 Silica (25 cm $\times$ 0.46 cm i.d.). The mobile phase consisted of 25 nM choline chloride in a solution of acetonitrile : methanol : water (47.5 : 47.5 : 5.0).

Results and Discussion

Exosurf recovered from various locations in the MLI

The total and relative amount of Exosurf recovered from the various stages in the MLI when delivered alone are shown in Figs 1 and 2, respec-

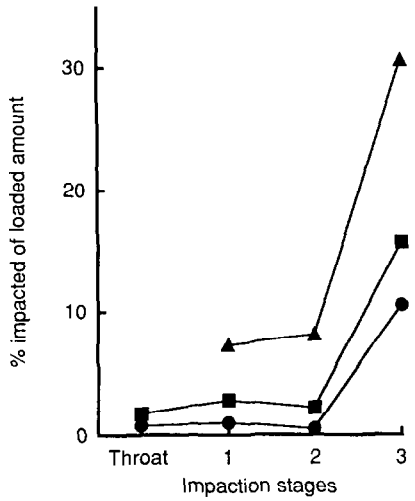

Fig. 1. The total amount of Exosurf impacted on the various stages on the MLI with increasing concentration of Exosurf in the donor suspension: (●) 2.2% w/v, (■) 0.22% w/v and (▲) 0.022% w/v Exosurf suspension.

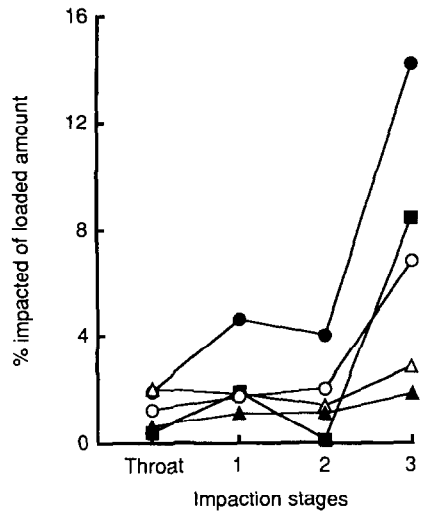

Fig. 3. The total amount of Fluoresbrite polybeads impacted on the MLI from a 0.25% suspension of beads with increasing particle size: (●) 0.35 μm, (■) 0.75 μm, (▲) 1.0 μm, (△) 2.0 μm and (○) 3.0 μm nominal size beads.

tively. Fig. 1 indicates that only a small fraction of the Exosurf in the nebulised suspension was recovered from the MLI and further that there is an inverse relationship between the concentration of Exosurf in the donor suspension and the percentage recovered in the MLI, probably due to the increased viscosity of high concentration suspensions. The amount of Exosurf delivered to stages 2 and 3 was 7.46, 2.42 and 0.42 mg for the 2.2, 0.22 and 0.022% suspension, respectively, per run, showing that the largest amount was still delivered per time unit when the highest concentration of Exosurf in the donor suspension had been used. Fig. 2 indicates that the same percentage of Exosurf impacted on the various stages irrespective of the concentration of Exosurf in the donor suspension and that by far the largest percentage of the delivered amount had an ECD of around 2 μm.

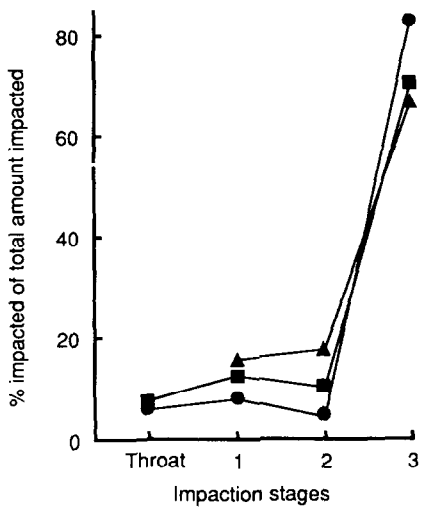

Fig. 2. The relative amount of Exosurf impacted on the various stages in the MLI with increasing concentration of Exosurf in the nebulised suspension: (●) 2.2% w/v, (■) 0.22% w/v and (▲) 0.022% w/v Exosurf suspension.

Polybeads recovered from the various locations in the MLI

The total amount of fluoresbrite polybeads recovered from the various stages in the MLI when delivered alone is shown in Fig. 3. Fig. 3 shows an inverse relationship between amount of impacted and particle size. When a suspension of particles is nebulised, several particles may cluster together in one droplet depending on the size of the droplets produced by the nebuliser, the relative sizes of the particles and the concentration of particles in the suspension (Gonda, 1985).

The droplet size distribution produced by the nebuliser will, therefore, to a large extent decide the amount of particles impacted on the MLI, and this possibly explains the low percentage of beads found in the MLI. However, it does not explain the differences observed in the total amount with respect to the size of the primary particles. One reason may be the formation of aggregates of particles which was seen in the nebulising chamber (Provasi et al., 1992). The aerodynamic diameter of an aggregate of particles is approximately equal to the cube root of the number of particles in the cluster times the diameter of the particles (Hickey et al., 1988). Using this equation indicates that aggregates of approx. 100 polybeads with a nominal size of 0.35 μm still would be able to reach the third stage, whereas aggregates of this number of particles for the other particle sizes would not be able to reach the last stage.

Polybeads recovered from the various locations in the MLI when aerosolised from mixtures of spheres and Exosurf

The total and relative amount of fluoresbrite polybeads recovered from the various stages in the MLI from mixtures of spheres and Exosurf are shown in Figs 4 and 5, respectively. Fig. 4 shows that the addition of Exosurf to the suspensions caused more particles to deposit in the MLI, this increase being least pronounced for the 0.35 μm spheres. Increasing the concentration of Exosurf caused an increasing amount of particles to impact in the MLI except for the 0.35 μm spheres. The increase was such that it was possible to deliver more fluoresbrite polybeads to stages 2 and 3 by using smaller particles. This finding indicates that the presence of Exosurf to some extent prevents aggregation of the spheres in the nebuliser allowing more of the larger particles to be carried in the nebulised plume. The relative percentages of particles impacted on the four stages in the MLI were remarkably similar for all sphere sizes. Fig. 5 therefore only shows the results for the 3.0 μm beads as an example of the results for all the bead sizes. The suggestion from Fig. 5 that the addition of Exosurf does not change the relative amount of spheres deposited

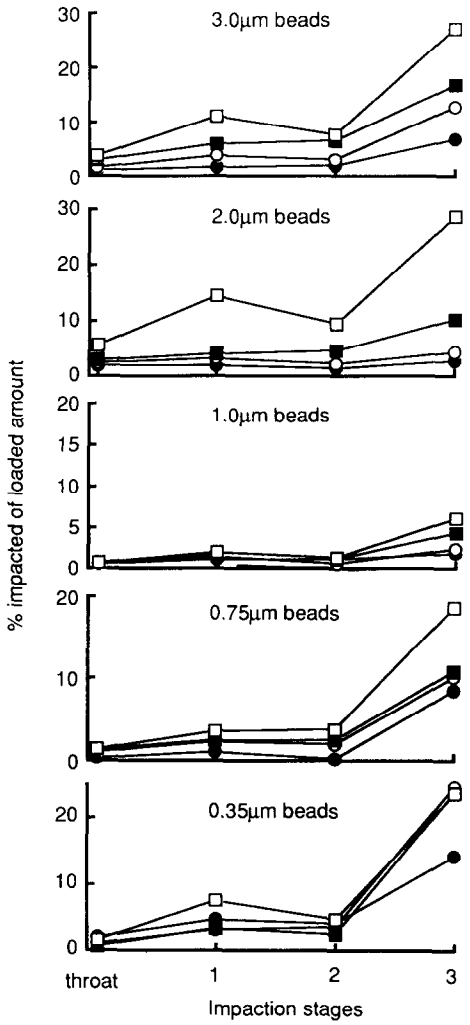

Fig. 4. The total amount of Fluoresbrite polybeads impacted on the various stages on the MLI with increasing concentrations of Exosurf in the donor suspension: (●) 0.0% w/v, (■) 0.022% w/v, (○) 0.044% w/v and (□) 0.22% w/v Exosurf suspension.

at each stage, together with the finding that more particles are deposited in the MLI when Exosurf is present in the suspension, indicate that a higher load of particles is carried in the nebulised droplets when Exosurf is present.

Exosurf recovered from the third stage in the MLI following nebulisation of Exosurf sphere mixtures

Table 1 shows the percentage of the loaded amount of Exosurf found in the third stage of the

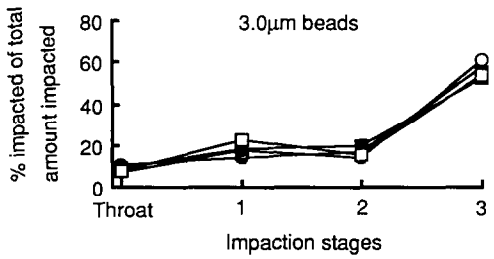

Fig. 5. The relative amount of Fluoresbrite polybeads impacted on the various stages on the MLI with increasing concentrations of Exosurf in the donor suspension: (●) 0.0% w/v, (■) 0.022% w/v, (○) 0.044% w/v and (□) 0.22% w/v Exosurf suspension.

MLI following nebulisation of suspensions containing 0.25% fluoresbrite polybeads with a size of 0.35, 0.75, 1.0 and 2.0 μm and 0.22% Exosurf. The results show that a similar amount of Exosurf impacted in the MLI irrespective of the nominal size of the beads. Comparing the results with those in Fig. 1 suggests that more Exosurf was nebulised from the mixture. This result is surprising in that one would expect the two types of particles to compete for space in the nebulised droplet. One hypothesis to explain the results is that the Exosurf particles are much smaller than the fluoresbrite polybeads and, therefore, can be packed in between the beads, another is that Exosurf coats the beads before nebulisation.

In conclusion, the study showed that particles of a larger average size could be aerosolised from an ultrasonic nebuliser when a surfactant also was present in the suspension for nebulisation and that using mixtures of particles and lung surfactant caused an increase in the amounts delivered of both within the range of 5–0.3 μm.

TABLE 1

The percentage of Exosurf impacted on the third stage of the MLI following nebulisation of suspensions containing 0.25% fluoresbrite polybeads with a size of 0.35, 0.75, 1.0 or 2.0 μm and 0.22% Exosurf

	Nominal sphere size (μm)			
	0.35	0.75	1.0	2.0
% Exosurf impacted on third stage of loaded amount	42.1%	54.0%	33.8%	28.1%

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