Spray Dried Powders and Powder Blends of Recombinant Human Deoxyribonuclease (rhDNase) for Aerosol Delivery[1]

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Received December 23, 1996; accepted January 14, 1997

Purpose. We have used rhDNase to investigate the feasibility of developing a dry protein powder aerosol for inhalation delivery.

Methods. Powders of rhDNase alone and with sodium chloride were prepared by spray drying. Powder blends were obtained by mixing (tumbling and sieving) pure rhDNase powder with 'carrier' materials (lactose, mannitol or sodium chloride). The weight percent of drug in the blends was between 5 and 70%. The particle size distributions and crystallinity of the spray dried powders were obtained by laser diffraction and X-ray powder diffraction, respectively. Particle morphology was examined by scanning electron microscopy. The ability of the powders and powder blends to be dispersed into respirable aerosols was measured using a Rotahaler™ connected to a multistage liquid impinger operating at 60 L/min.

Results. Pure rhDNase powder was quite cohesive with a fine particle fraction (FPF or 'respirable fraction': % wt. of particles < 7 μm in the aerosol cloud) of about 20%. When particles also contained NaCl, the powders were dispersed better to form aerosols. A linear relationship was observed between the NaCl content and FPF for a similar primary size (~3 μm volume median diameter) of particles. The particle morphology of these powders varied systematically with the salt content. For the blends, SEM revealed a monolayer-like adhesion of the fine drug particles to the carriers at drug contents ≥50 % wt. An overall 2-fold increase in FPF of rhDNase in the aerosol cloud was obtained for all the blends compared to the pure drug aerosols.

Conclusions. The aerosol properties of spray dried rhDNase powders can be controlled by incorporation of a suitable excipient, such as NaCl, and its relative proportion. Coarse carriers can also enhance the performance of rhDNase dry powder aerosols.

KEY WORDS: rhDNase; dornase alpha; powder aerosol; spray drying; inhalation; powder blends.

INTRODUCTION

Recombinant human deoxyribonuclease (rhDNase, dornase alpha) is the first recombinant human protein approved for therapeutic use by inhalation (1–5). It is currently administered by nebulization of 2.5 mL of a 1 mg/mL aqueous protein solution. rhDNase is an excellent model protein: it is a hydrophilic glycosylated molecule, MW ~33 kDa. It is relatively resistant to interfacial denaturation. These properties make it a good representative of a wide class of therapeutic proteins that may be considered for administration by inhalation either as aqueous solutions, or as a spray-dried powder. Other proteins, such as the human growth hormone, may require more sophisticated processing if they have a tendency to undergo denaturation at the air-water interface (6).

We have studied the feasibility of developing a dry powder delivery system for rhDNase. The performance of a dry powder system depends on both the aerosol device and the powder properties (7,8). To generate respirable aerosols, powder formulations must meet two opposing criteria: the particles have to be sufficiently fine (e.g. < 7 μm) for lung deposition, and yet coarse enough for optimal flow in device filling and emptying (7–9). Thus, powders are frequently pelletized as loose agglomerates of the drug particles (e.g. Terbutaline in Turbuhaler®), or blended with 'coarse' inert carriers (7,8,10,11). Physical blending of drug with a coarse carrier is a classic technique employed in inhalation products (10). In an ideal drug-carrier system, the adhesion of the drug to the carrier particles is strong enough to prevent demixing during filling, handling and storage, but not so strong as to prevent the generation of fine drug particles by detachment from the carrier during inhalation. Factors affecting the performance of a blend include the ambient conditions, process conditions, as well as drug and carrier properties (11,12).

In the present study, we have concentrated on the powder dispersion and not on the bulk flow properties. A low resistance device (Rotahaler) was used to study the dispersion of the rhDNase powders. The relatively simple and gentle mechanism for capsule emptying and powder deagglomeration in this device was attractive for this study with the view to run tests that would discriminate between powders with different cohesive and adhesive properties. Rotahaler also represents a "low resistance" device that could be readily used by patients with impaired lung function at the inspiratory flow rate of 60 L/min used in this study (13).

The powders were obtained by spray drying (6). Sodium chloride was chosen as an excipient as it is already present in the current product for rhDNase (Pulmozyme® nebulizer solution) (1–5). Further, we attempted to improve the dispersibility of the pure rhDNase powder by blending it with various carriers. It must be emphasized that we were not trying to minimize device retention, but rather we were investigating the dispersing properties of various formulations. We focused on the effect of the carrier type and the drug to carrier ratio.

MATERIALS AND METHODS

Preparation of rhDNase Powders and Powder Blends

rhDNase powders were obtained by spray drying (Büchi, 190 Mini Spray Dryer). The rhDNase solutions (15–90 mg/mL) were fed at a rate of 5 mL/min. The inlet and outlet air temperatures of the spray dryer were 90 and 55°C, respectively. Details of the rhDNase and excipient contents in the powders are presented in Table 1. The carriers were lactose (Pharmatose 200 M and DCL 11, DMV, WI), D-mannitol (Sigma, MO), and

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0724-8741/97/0400-0431$12.50/0

Table 1. Content of Spray Dried Powders of rhDNase with NaCl

DNase[a] (wt.%)	Water (wt.%)	NaCl[b] (wt.%)	Monomer (%)	Activity[c]
10.5	1.3	88.5	96.5	ND
35.3	4.5	60.8	98.2	114[d]
47.6	5.5	45.7	99.5	ND
68.1	4.6	27.5	99.7	ND
82.2	10.1	8.1	99.5	105[e]

[a] rhDNase (glycosylated) content determined by UV absorption at 280 nm using an absorptivity of 1.4 $(mg/mL)^{-1}cm^{-1}$.
[b] NaCl content determined by flame photometry (Model IL943, Instrumentation Laboratory).
[c] Activity was determined by the methyl green assay normalized to the protein concentration measured by UV absorption (15). ND: not determined.
[d] 114 ± 2%, number of determination n = 8.
[e] The mean of two determined values of 109.8 and 100.7%.

sodium chloride AR (Mallinckrodt, KY). The particle sizes of lactose and mannitol were determined by sieving (USA standard testing sieves, Gilson, OH). Due to a limited amount of material, particle sizing of sodium chloride was performed by laser diffraction as described below.

Blends of pure rhDNase powder with the carrier powders were prepared in the following sequence of steps: i) Weighing the carrier into 3 mL glass vial. ii) Weighing the pure rhDNase powder and adding it to the carrier in the vial. iii) Manual (end over end) tumbling the vial for 5 min (mixing). iv) Sieving the blend through a 250 μm mesh (breaking up any *large* agglomerates). v) Return of the powder mix into the vial and repeating the tumbling & sieving step. The total weight of all the blends was fixed at 300 mg. A sample (10–40 mg) was taken from each blend to check the protein content by UV absorbance at 280 nm (the carriers do not absorb significantly at this wavelength) and was found to be close (≥ 90 %) to the value based on the weight added. The effect of blending on the dispersing properties of pure rhDNase powder was also studied and was found to have no significant impact.

Scanning Electron Microscopy (SEM)

rhDNase particles and the blends were coated with platinum (thickness 10 nm) under partial vacuum (0.07 torr with argon gas) on a Hummer sputter coater (Anatech). The samples were examined by SEM (model 525M, Philips, Holland) operating at 5 kV and equipped with a secondary electron detector.

X-ray Powder Diffraction

Pure spray-dried rhDNase powder is amorphous [indeed, very specialized methods need to be generally used to make even minute quantities of crystalline proteins (14)]. X-ray powder diffraction was used to monitor the crystallinity of the powders containing sodium chloride. The samples were analyzed on an X-ray diffractometer (D/max-B, Rigaku, Tokyo) using CuK radiation, 35 kV and 15 mA, angular increment of 0.02°/s with an increment count time of 3 s. Crystallinity of the rhDNase-NaCl powders was quantified using an internal reference standard (silicon powder, 640b, National Bureau of Standards, USA) and expressed as the relative intensity of the NaCl diffraction peak at 2θ angle of 31.7° to that of silicon powder at 28.4°. 40 mg rhDNase-salt powders were mixed with equal amount of the standard material by tumbling in a small tube. The sample was then packed on a glass sample plate with a rectangular cavity of dimensions 16mm × 20mm × 0.2mm and run in duplicate.

Moisture Content Determination

Samples of ~5 mg were placed in platinum pans and heated at a rate of 4°/min under a nitrogen purge (~30 mL/min) in a thermogravimetric analyzer (TGA 7, Perkin Elmer) linked to a data station (Model 7700, Perkin Elmer). % moisture was calculated as the weight loss between room temperature and 150°C where the profiles leveled off.

Evaluation of rhDNase Integrity in the Spray Dried Powders

The spray dried powders were reconstituted in de-ionized water to 1 mg/ml for bioactivity and aggregation assays described elsewhere (1–4,15).

Particle Sizing

The particle size distribution of the spray dried powders was determined in liquid suspensions by laser diffraction (MasterSizer, Malvern Instruments). Each powder was dispersed in ethanol or isopropyl alcohol containing Tween 80 (approximately 1% v/v) using a small ultrasonic bath. An aliquot of the suspension was added to the diffractometer's liquid sample cell containing ethanol filtered through a 0.45 μm filter (Millipore). The suspension concentration was adjusted to attain optimal obscuration. Particle size analysis was performed using presentation standard and model-independent fitting routine (Software version BD.01, Malvern Instruments). Measurements were repeated 5 to 10 minutes apart to ensure that no dissolution or agglomeration of the powders occurred. The size distribution was expressed in terms of the volume median diameter (VMD) and span. VMD is the diameter below which 50% by volume of the particles reside. VMD is directly related to the mass median diameter (MMD) by the density of the particles (provided that all the particles have the same size-independent density). Span is a measure of the width of the volume (or mass) distribution relative to the median diameter. Span = $[D(v,90)-D(v,10)]/D(v,50)$, where D(v,90), D(v,10) and D(v,50) are the equivalent volume diameters at 90, 10 and 50% cumulative volume, respectively. The aerodynamic diameters are obtained by multiplying the mass-based diameters with the square root of density (7). The latter was measured by air displacement pycnometry (Beckman, Model 930) for two powders for which sufficient amount of material was available.

Measurement of Aerosol Properties

The critical properties measured in these studies are defined in the Appendix. The dispersing behavior of each powder as an aerosol was assessed in a powder inhaler (Rotahaler®, Allen & Hanburys) coupled to a multiple stage liquid impinger (AB Draco). From experience, the capsule emptying in the Rotahaler is achieved readily, making the device performance

in other respects only weakly dependent on the powder flow from the capsule. The device has a low air flow resistance and the powder is dispersed simply through the grid in the mouthpiece. A known volume of pure water was pipetted onto each stage of the impinger to wet the collection surfaces. The air flow rate was adjusted to 60 ± 2 L/min using a mass flow meter (Sierra Instruments) via a vacuum source. Each powder was weighed (20–40 mg) into five gelatin capsules (size no.3, Torpac). Each capsule was then snapped open inside the Rotahaler which was then pushed into the throat of the 'running' impinger. The powder was aerosolized by the air flow and drawn into the impinger. After all five capsules had been discharged, the impinger was agitated to ensure dissolution and homogeneity of the solution on each stage. The inhaler, emptied capsules, throat and filter of the impinger were washed quantitatively. Solution aliquots from each location were withdrawn for assay of protein content by UV absorption at 280 nm using an absorptivity of 1.6 cm^{-1} $(mg/mL)^{-1}$. Similarly five gelatin capsules, each containing 40–50 mg of the blends, were emptied and dispersed by the Rotahaler. Thus, for each blend, 70–80% of the total 300 mg was analyzed.

RESULTS AND DISCUSSION

rhDNase Powders

Pure rhDNase has identical morphology observed by SEM as the 8% NaCl sample (Figure 1). As the NaCl content increases, the morphology changes from spheres with a smooth surface texture, to spheres with surfaces having numerous apparently cubic salt crystals, to faceted spheroids and finally to spheroidally agglomerated crystals.

At a high sodium chloride level (~90 wt.%), the water content is low (~1 wt.%, Table 1) due to the anhydrous nature of NaCl crystals. At a low sodium chloride level (< 10 wt.%), the powder has relatively high water content (~10 wt.%), owing to the water association with the protein. Those powders with intermediate NaCl levels have similar water contents of approximately 5 wt.%. Thus, the residual water content does not vary linearly with the powder composition. Two of the freshly prepared powders were found to be fully active and show only minimal protein aggregation (Table 1) but no implications about the long term stability can be drawn yet from this data.

Figure 2 compares the particle size distribution of the original powder (as determined by laser diffraction in suspension) to that in the aerosol cloud generated by the Rotahaler (as determined by the multiple stage liquid impinger) for two powders containing high and low salt contents. The aerodynamic diameter of the raw powders was obtained using a density of 1.3 and 2.1 g/cm^3 for the 8% and 88% sodium chloride sample, respectively. The data for the aerosolized powders indicate that the powders were not sufficiently dispersed to recover the size distribution of the original particles. This is a result of the cohesiveness of the powder and the inadequate dispersing efficiency of the device (10,16–18). As shown in Figure 2, the 8% sodium chloride powder is not as well dispersed as the 88% sodium chloride powder, reflecting the fact that the 8% powder is more cohesive (see below).

Effect of the Excipient Content

A linear relationship was found between the fine particle fraction (FPF) of rhDNase powders, the NaCl content and the crystallinity (Figure 3). Crystalline powders of rhDNase containing high NaCl content are dispersed better than the amorphous or less crystalline ones with low salt content. The bulk particle shape is apparently unimportant since the faceted and smooth particles were both spheroidal, yet their dispersing properties were very different. This may be explained to be the result of a difference in the surface properties that affect inter-particle forces. It is known that amorphous solids generally have higher surface energy giving rise to direct attractive forces between particles and to adsorption of water leading to capillary inter-particle force (19).

Effect of Primary Particle Size

Figure 4 summarizes the powder dispersion as a function of the primary particle size and composition. The effect of particle size is related to cohesion which is a measure of the force needed to separate a unit mass of particles from their stable agglomerated state. Contributions from capillary force and electrostatic interactions to cohesion are uncertain in the present study. If, as an approximation, cohesion is taken as the van der Waals force per unit mass of particles, then it is inversely proportional to the square of the particle size (20,21). Thus, finer particles are more cohesive and difficult to disperse. As the primary particles become bigger, they are easier to be dispersed due to a decrease in cohesiveness, but the available fraction of 'fines' (assuming complete dispersion) will decrease. Thus, the maximum fine particle fraction in the aerosol is the results of a balance of the available fine particles against the particle cohesion. The powders of 8% and 88% NaCl generally follow the trend just described. The optimum particle size for maximum respirable fraction shifts from an estimated value of ≥5.8 μm for the 8% sodium chloride powder to ≤3–4 μm for the 88% sodium chloride powder.

Powder Blends

The mass median diameter (MMD) of the various carriers were measured as 43, 87, 42 and 115 μm with geometric standard deviations (GSD) of 1.5, 1.7, 1.5 and 1.6 for mannitol, NaCl, lactose 200M and lactose DCL 11, respectively. The value of GSD was calculated as the mean of the ratios 84% undersize/50% size and 50% size/16% undersize since the size distributions were not perfectly log-normal.

SEM Morphologic Examination of rhDNase Blends

The 200 M lactose particles appeared irregular in shape (Figure 5). In the blends with ≥50% rhDNase, an apparent monolayer type adhesion of the fine rhDNase particles on the carrier was observed. At low blend ratio, the monolayer type of adhesion is less likely to be seen since more carrier particles are available for the drug particles to adhere (this is in agreement with estimates obtained by division of the average surface area of the carriers by the average projected area of the rhDNase particles).

The mannitol particles are elongated compared to lactose. SEM reveals that adhesion of rhDNase particles to mannitol is less efficient (Figure 5). In addition, 'monolayer adhesion' is not obvious even in the blend containing 50% rhDNase.

Ground sodium chloride crystals are irregularly shaped. The particulate adhesion is similar to that observed in the lactose

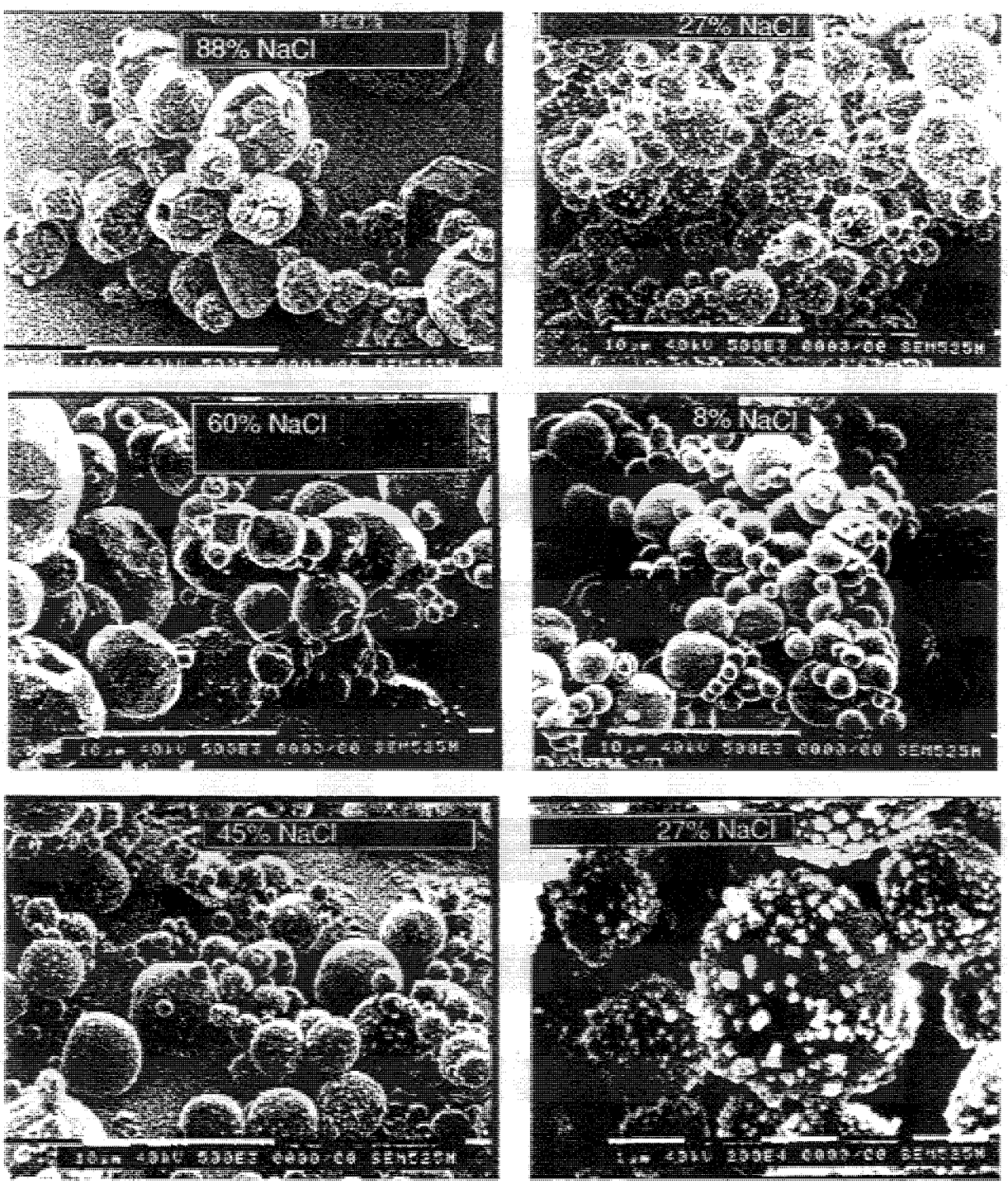

Fig. 1. Scanning electron micrographs of rhDNase particles co-spray dried with NaCl (pure rhDNase looks identical to the 8% NaCl sample; the micrograph is omitted).

blends, i.e., apparent monolayer adhesion occurs in the blend containing 50%, but not 10%, rhDNase (Figure 5).

Effect of the Carriers on Dispersion

A summary of the results is given in Table 2. Compared to pure rhDNase, lactose blends improve the fine particle fraction (FPF) to around 50% (Figure 6), irrespective of the lactose type, carrier particle size, or the rhDNase content in the blend. The particle size distribution of the rhDNase particles in the aerosol generated from the blends was closer to the primary particle size distribution of pure rhDNase powder than that of the aerosol generated from the pure rhDNase alone (plots not shown). The dispersing efficiency of the lactose blends was 3-

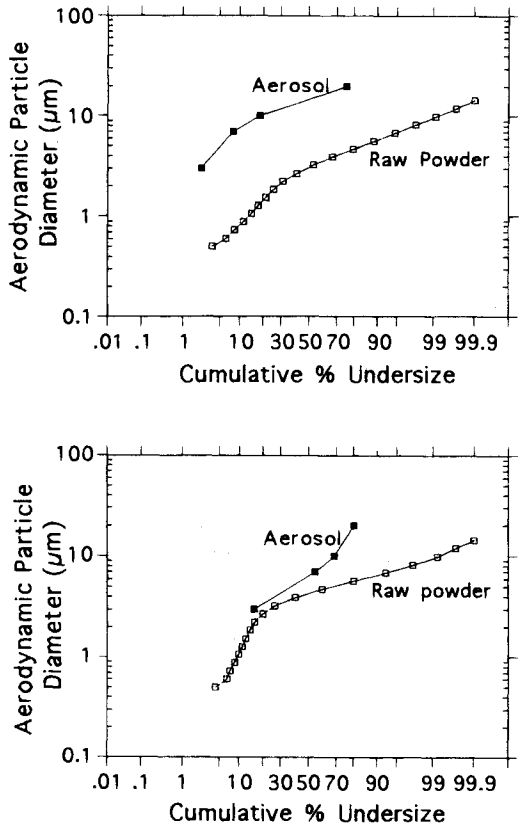

Fig. 2. Comparison of the particle size distribution of the powders before and after aerosolization by the Rotahaler for powders containing low (8%, top) and high (88%, bottom) salt contents in the co-spray dried particles with rhDNase.

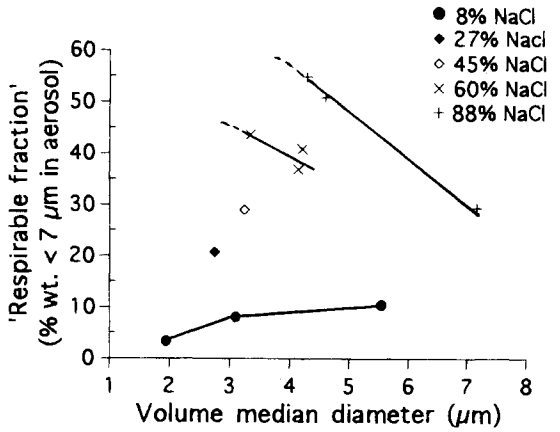

Fig. 4. A summary of the dispersing properties (as 'respirable fraction' or FPF) versus particle size for powders containing co-spray dried NaCl with rhDNase.

to 4-fold times greater than that of the pure rhDNase powder as a result of the 2-fold increase in FPF and a 1.5–2-fold decrease in the device retention (Figure 6). A similar aerosol performance improvement was also observed for the lactose blends containing 5–10% pure rhDNase powder with a 'large' (5.5 μm) or 'small' (2.6 μm) mass median diameter, indicating the relative insensitivity of the blend performance to the protein particle size. This may be explained by the inability of Rotahaler

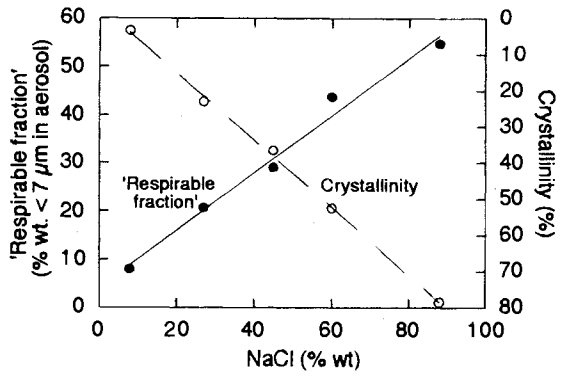

Fig. 3. Relationship between NaCl content, the corresponding crystallinity (pure rhDNase powder is amorphous) and the dispersing properties (as 'respirable fraction' or FPF) of rhDNase powders [all powders had similar primary particle size distributions before aerosolization with median diameters of 2.7–3.3 μm (span 1.04–1.63)].

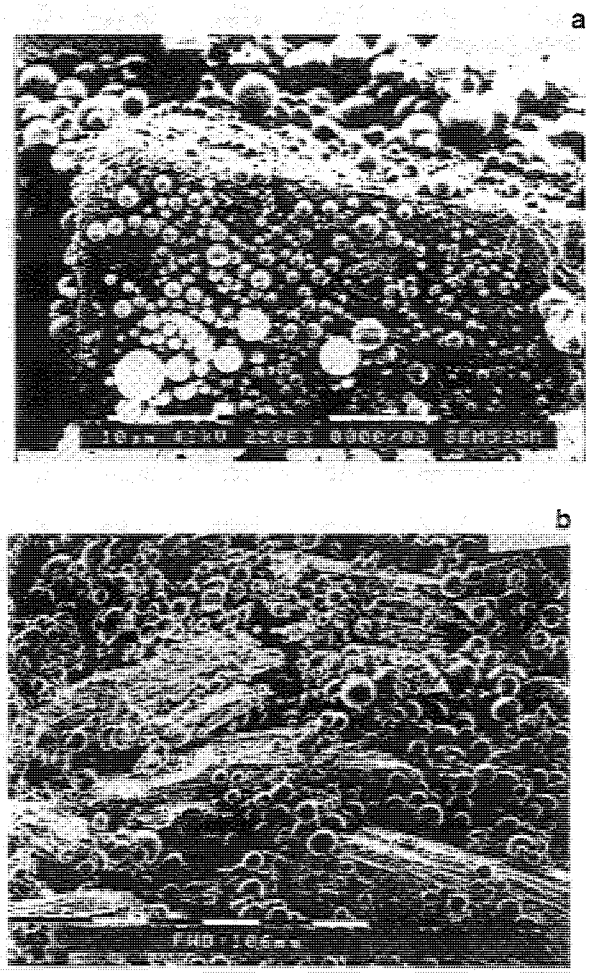

Fig. 5. Scanning electron micrographs showing adhesion of pure rhDNase particles to 200M lactose (a), mannitol (b) and sodium chloride (c).

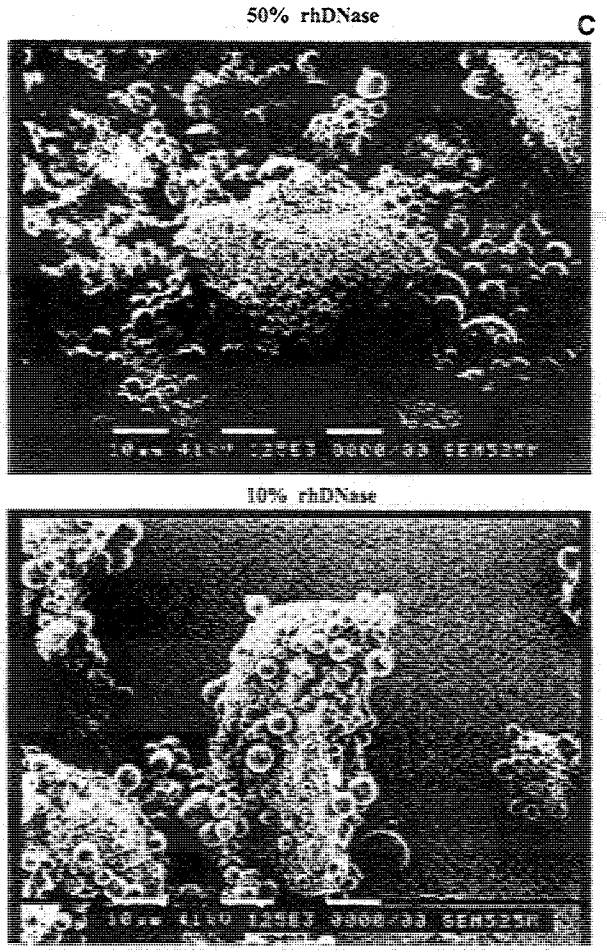

Fig. 5. Continued.

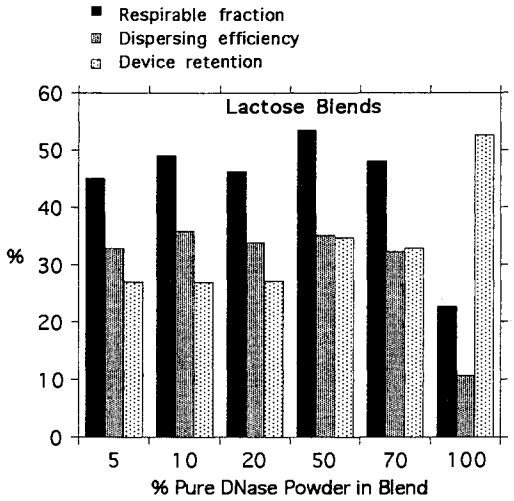

Fig. 6. Dispersion properties of lactose blends containing different proportions of pure rhDNase particles.

to break up below a certain size these highly cohesive pure protein powders: they show a similar low FPF of about 20% (17–23%) despite the fact that there is about twofold difference in the primary particle size between the two pure protein powders.

Mannitol blends show an almost identical FPF to the lactose blends and a tendency for slightly higher device retention compared to the lactose blends. For the sodium chloride blend, the FPF is dependent on the rhDNase content in the blends. When the blend contained a larger proportion of rhDNase (50%), FPF was increased about 2-fold. However, the device emptying did not improve as much as in the sugar blends, so the overall dispersing efficiency only increased by a factor of 2.5 compared to the pure rhDNase powders (instead of 3–4-fold overall improvement in the lactose blends). When the blend contained only a small proportion of rhDNase (10%), there was no improvement in the FPF over the pure rhDNase powders. Such a lack of improvement may not be due to incomplete detachment of the rhDNase particle from the carrier during dispersion since the FPF did not decrease compared to the unblended rhDNase powder. The complexity of the mechanism of generation of fine particles of rhDNase during aerosolization of the blends is illustrated by the results with the sugar carriers: apparent monolayer adhesion occurs with lactose but not with mannitol, yet aerosol performance improved with both. Particulate cohesion and adhesion, shear force and inter-particle collisions are likely to be the dominant players controlling the dispersion efficiency. It is the relative magnitude of these formulation and device-dependent variables that needs to be adjusted for optimum inhalation powder delivery performance. For proteins, the formulation development is generally more complex due to the greater sensitivity of protein molecules to inactivation and denaturation during processing and storage, as compared to small molecules.

CONCLUSIONS

Pure rhDNase powder was quite cohesive as evidenced by the low fine particle fraction in the aerosol. Respirable rhDNase powders can be designed by co-spray drying rhDNase with suitable excipients varying the protein-excipient composition, and through the use of physical blends of fine particles containing rhDNase with suitable coarse size carriers.

The presence of NaCl in rhDNase changed the morphology and crystallinity of the co-spray dried powders, and this was paralleled by improvement in the fine particle fraction of rhDNase. In the blends with lactose in particular, the rhDNase particles adhered apparently in the form of a monolayer to the carrier. Within the range of parameters studied, the improvement, compared to the unblended pure rhDNase powder, was found to be relatively independent of the carrier types, the rhDNase/carrier blend ratio, and the primary particle size distribution of the pure rhDNase particles (2.6–5.5 μm).

ACKNOWLEDGMENTS

We thank the following scientists who, at the time this work was carried out, worked at Genentech, Inc.: Rodney Pearlman for advice and support, Andy Walsh for spray drying the rhDNase, Mark Siegel for SEM, Assay Services and David Cipolla for bioassay support. Joel Sparks from Stanford University has generously provided the X-ray powder facility. We are also very grateful to the reviewers and editors for valuable comments that resulted in a much improved manuscript.

Table 2. Summary of Aerosol Performance

Carrier	Mass median diameter of DNase powder (μm)[c]	DNase in blend (% wt.)	Capsule loading (mg)	DNase dose (mg)	Respirable dose (mg)
No carrier	5.5	—	24.9	24.9	2.44
(pre-blending)	2.6	—	21.9	21.9	2.34[a]
	3.1	—	20.0	20.0	1.84
Lactose	5.5	7	42.9	3.00	1.05
(200 M)		10	45.3	4.53	1.65
	2.6	5	51.4	2.57	0.85[b]
		10	42.7	4.27	1.53
		20	41.8	8.36	2.83
		50	42.3	21.2	7.40[a]
		70	45.0	31.5	10.1
Lactose	2.6	10	52.3	5.23	1.59
(DCL 11)					
Mannitol	2.6	10	51.9	5.19	1.97
		50	22.5	11.25	3.13
NaCl	3.1	10	50.1	5.01	1.00
		50	22.3	11.15	2.69

[a] To compare the respirable dose for the pure powder vs blends at the same DNase loading to the device.
[b] To compare the respirable dose from a 2.5 mg rhDNase loading based on currently approved nebulizer formulation solution.
[c] The span of the size distribution for all three rhDNase powders was 2.0 ± 0.1, span = [D(v,0.9)− D(v,0.1)]/D(v,0.5), where D(v,0.9), D(v,0.1) and D(v,0.5) are the equivalent volume diameters at 90, 10 and 50% cumulative volume, respectively, as measured by the light diffraction technique using the Malvern MasterSizer.

APPENDIX

Definition of Terms Used to Characterize Powder Dispersion

All particle sizes in this Appendix refer to the aerodynamic diameter (7).

Fine particle fraction (FPF) (sometime also referred to as 'respirable fraction') is the % of drug in particles smaller than 7 μm in the aerosol cloud.

'Device retention' is the % mass of drug retained in the powder inhaler (and capsules) at the end of drug administration.

Fine particle dose (called by some authors 'respirable dose') is the mass of drug in particles smaller than 7 μm in the aerosol cloud.

'Dispersing efficiency' is the mass of drug in particles smaller than 7 μm in the aerosol cloud divided by the initial drug load in the aerosol device.

Note that 7 μm is simply an arbitrary cutoff diameter of the liquid impinger and does not necessarily imply the real "cut-off" size needed for deposition of rhDNase in the respiratory tract.

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