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Physicochemical Compatibility of Mixtures of Dornase Alfa and Tobramycin Containing Nebulizer Solutions

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Summary. Patients suffering from cystic fibrosis (CF) often need to inhale multiple doses of different nebulizable drugs per day. Patients attempt to shorten the time consuming administration procedure by mixing drug solutions/suspensions for simultaneous inhalation. The objective of this experimental study was to determine whether mixtures of the nebulizer solution dornase alfa (Pulmozyme®) with tobramycin nebulizer solutions (TOBI® and GERNEBCIN® 80 mg) are physico-chemically compatible. Drug combinations were prepared by mixing the content of one respule Pulmozyme® with either one respule TOBI® or one ampoule GERNEBCIN® 80 mg. Test solutions were stored at room temperature and exposed to light. Dornase alfa activity and tobramycin concentrations were determined by using a kinetic colorimetric DNase activity assay and a fluorescence immunoassay, respectively. Physical compatibility was determined by visual inspection and measurements of pH and osmolality. Tobramycin concentration was not affected by mixing the drug products. In spite of the high variability of the dornase alfa potency assay, it is obvious that activity is especially affected by sodium metabisulfite, used as excipient in GERNEBCIN®. Patients should be advised, not to mix Pulmozyme® with GERNEBCIN® because of the incompatibility reaction. Further analytical studies are needed in order to determine the integrity and activity of dornase alfa in mixtures of Pulmozyme® with TOBI®. Finally clinical studies are necessary in order to demonstrate equivalent efficacy and safety of simultaneous inhalation in comparison to consecutive inhalation of both drugs. **Pediatr Pulmonol. 2009; 44:134–141.** © 2008 Wiley-Liss, Inc.

Key words: cystic fibrosis; multiple drug inhalation solution; compatibility; sodium metabisulfite; dornase alfa; tobramycin.

INTRODUCTION

Chronic pulmonary infections are the major cause of morbidity and mortality in patients suffering from cystic fibrosis (CF). Both short and long-term studies indicate that dornase alfa (recombinant human deoxyribonuclease) therapy improves pulmonary function in selected patients with CF. Dornase alfa is to be administered by oral inhalation via nebulization. The usual dosage is 2.5 mg (2.5 ml of undiluted solution) once or twice daily. Nebulizers convert drug solutions or drug suspensions by ultrasound or a jet stream of compressed air into an aerosol. Aerosolized droplets or particles should be 1–5 μm in diameter to ensure that the droplets reach bronchioles.[1]

Generally multiple drug inhalation therapies are indicated in CF patients. Antibiotics, that is, tobramycin or colistin, bronchodilators, that is, albuterol and ipratropium, and corticosteroids, that is, budesonide or fluticasone are used as adjunctive therapy. Patients often need to inhale multiple doses of the different drugs per day. Each nebulization procedure takes about 15 min. Thus, patients tend to mix drug solutions or suspensions for simultaneous nebulization. In order to help patients making the best use of their inhalation drugs, knowledge of the compatibilitiy of drug solutions and suspensions in inhalation cups for oral inhalation is a prerequisite. However available data are limited.[2]

The official prescribing information and the relevant monografs in the AHFS Drug Information reference

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Pulmozyme®, Tobi®, and Gernebcin® were kindly provided by Roche, Chiron, and Infectopharm, but the companies had absolutely no role in the conduct of the experiments. There is no conflict of interest for the authors with the facts presented.

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book[3] for Pulmozyme® (brand of dornase alfa), TOBI® and GERNEBCIN® (brands of tobramycin licensed for inhalation therapy in CF patients) state that the inhalation solutions should not be diluted or mixed with other drug solutions in nebulizers. Drugdex[4] even includes a warning against mixing tobramycin and dornase alfa (Pulmozyme®), but no reference is given. To our knowledge there are no published data concerning the compatibility of Pulmozyme® with other nebulizable drugs. The objective of this study was to determine whether mixtures of Pulmozyme® inhalation solution with TOBI® or GERNEBCIN® inhalation solution are physico-chemically compatible. Test solutions were prepared by mixing clinical relevant doses of brands. Dornase alfa activity and tobramycin concentrations were determined by using a kinetic colorimetric DNase activity assay and a fluorescence immunoassay, respectively. Physical compatibility was determined by visual inspection and measurements of pH and osmolality. The results of the compatibility studies can be used to inform patients and health care personnel, if mixing of dornase alfa and tobramycin formulations in nebulizer cups and simultaneous inhalation is feasible. The data presented here have in part been published previously in abstract form.[5] In the meantime data reporting the physicochemical compatibility of nebulizable drug mixtures containing dornase alfa and ipratropium and/or albuterol were published.[6]

METHODS

Sample Preparation

All tests were performed with the commercially available nebulizer solutions Pulmozyme®, TOBI®, and GERNEBCIN®. Mixtures were prepared by mixing 2.5 ml of Pulmozyme®, withdrawn from a 2.5 ml respule containing 2,500 U Dornase alfa dissolved in sodium chloride solution and containing calcium chloride to ensure stability activity of Dornase alfa[7,8] with either 5.0 ml of TOBI®, withdrawn from a 5.0 ml respule containing 300 mg tobramycin dissolved in 5 ml sodium chloride solution,[9] or with 2.0 ml of GERNEBCIN® 80 mg, withdrawn from a 2.0 ml ampoule containing tobramycin-2.5-sulfate equivalent to 80 mg tobramycin base and sodium metabisulfite as excipient.[10] Mixtures were prepared in polystyrene or glass containers, gently mixed and stored at room temperature under ambient light conditions (mixed daylight and normal laboratory fluorescent light).

Determination of dornase alfa activity was performed on test solutions stored in polystyrene-containers. All over 9 test solutions of Pulmozyme® mixed with TOBI® and 12 test solutions of Pulmozyme® mixed with GERNEBCIN® were prepared on different days. Samples containing nominal 25 U dornase alfa (i.e., 100% activity) were withdrawn from each test solution immediately after mixing and after 1–2, 7, 24, 28–29, and 33–34 hr of storage. Samples were diluted with buffer C (25 mM Hepes, 4 mM $CaCl_2$, 4 mM $MgCl_2$, 0.1% BSA, 0.01% Thiomersal, 0.05% Tween® 20, pH 7.5), in order to fit the calibration curve and were assayed in quadruplicate immediately. Thereby samples were first diluted to a volume of 10 ml and an aliquot of 500 µl was further diluted 1:10. Each dilution step was completed by vortexing.

Dornase alfa activity was also determined in triplicate for admixtures of Pulmozyme® with 0.05% sodium metabisulfite solution. Five hundred microliters aliquots of undiluted Pulmozyme® inhalation solution were added to 400 µl 0.05% sodium metabisulfite solution (dissolved in 0.9% NaCl solution) in polystyrene-containers. Samples containing nominal 25 U dornase alfa (i.e., 100% activity) were withdrawn from each test solution immediately after mixing and after 1–2, 20 and 28–29 hr of storage and further diluted.

Determination of tobramycin concentrations was performed on test solutions stored in glass containers. Test solutions were prepared in triplicate and 10 µl samples were withdrawn immediately after mixing and after 8 hr of storage. Samples were diluted with commercially available fluorescence immunoassay buffer (using a Microlab® 500 diluter, Hamilton) in order to fit the calibration curve and were assayed in duplicate immediately. Dilution resulted in a nominal tobramycin concentration of 8 µg/ml. Each dilution step was followed by vortexing.

Samples of pure Pulmozyme® inhalation solution or 2 ml GERNEBCIN® solution mixed with 2.5 ml 0.9% NaCl solution, and diluted with buffer C, were assayed as control solutions.

Assays

Dornase Alfa Activity

Enzymatic activity of dornase alfa was determined by using a kinetic colorimetric DNase activity assay developed from Lichtinghagen.[11] Degradation of DNA-methyl green substrate by dornase alfa was determined by measuring ΔA/min at 600 nm and 37°C using the Cobas Mira (Roche Diagnostics, Mannheim, Germany) automated analyzer. Ninety microliters samples of the diluted test solutions were transferred into a sample tube of the analyzer containing 90 µl DNA-methyl green substrate-solution diluted with 100 µl buffer C. The colorimetric substrate-solution was prepared by mixing 10 ml DNA-solution (0.5 g DNA in 250 ml buffer A) with 600 µl methyl green-solution (0.4 g methyl green in 100 ml buffer B), 2,396 µl buffer C and 10 µl H_2O_2 35% in order to remove free methyl green. Buffer A consisted of 25 mM Hepes and 1 mM EDTA, pH adjusted to 7.5. Buffer B contained 20 mM sodium acetate pH adjusted to

4.2. According to Lichtinghagen[11] the substrate solution was always freshly prepared and preincubated overnight at room temperature. For each assay, calibration was performed with dilutions of nominal 0%, 20%, 40%, 60%, 80%, 100%, 120% dornase alfa activity. Quadruplicate determination of control and mixture samples was performed by assaying each sample once, and threefold repetition of the analysis in the identical sample order.

Assay precision was determined by analyzing Pulmozyme® solutions on different days. Samples containing nominal 100% or 85% dornase alfa activity yielded a mean dornase alfa activity of $89 \pm 8.3\%$ ($n = 15$) or $78 \pm 9.3\%$ ($n = 39$).

Diluted tobramycin nebulizer solutions (GERNEBCIN®, TOBI®) as well as diluted sodium metabisulfite solutions showed no enzymatic activity.

Samples with nominal dornase alfa activities $\geq$85% (mean), corresponding to measured dornase alfa activities $\geq$78% (mean), were defined as compatible with regard to the drug substance dornase alfa.

Tobramycin Concentration

Analysis of tobramycin concentrations was performed using a commercial fluorescence immunoassay (TDx/TDxFLx, Abbott, Wiesbaden, Germany). The assay was conducted on a TDx/TDxFLx system (Abbott).

The assay was validated as stability-indicating by analyzing forced-degraded tobramycin solutions. Aqueous solutions of TOBI® and GERNEBCIN® were degraded with H_2O_2 35% at 80°C for 8 hr. Tobramycin concentrations declined to 25% of the nominal concentrations and the initially measured pH 5 to pH 4, respectively. Visible changes were not observed.

The calibration curve was determined by using GERNEBCIN® (brand of tobramycin nebulizer solution) diluted with sodium chloride 0.9% (1:1). Aliquots were diluted with commercial fluorescence immunoassay buffer to nominal tobramycin concentrations of 0, 0.5, 1.5, 3.0, 6.0, 10.0 μg/ml. Control samples were prepared by dilution of GERNEBCIN® to nominal concentrations of 4 and 8 μg/ml. Control samples were stored under refrigeration and measured in duplicate within each assay.

Assay precisions were determined with mixtures of 2 ml GERNEBCIN® and 2 ml 0.9% NaCl solution. Samples were further diluted with commercial fluorescence immunoassay buffer to a nominal concentration of 8 μg/ml. Duplicate determinations of six separately prepared samples (intraday precision) resulted in a mean tobramycin concentration of 7.7 μg/ml $\pm$ 3.7% (rel. SD). Duplicate determinations of 7 separately prepared samples on 7 different days (interday precision) yielded a mean tobramycin concentration of 7.7 μg/ml $\pm$ 4.8%. Assaying pure Pulmozyme® nebulizer solutions tobramycin concentration was 0.0 μg/ml.

Samples with tobramycin concentrations $\geq$90% (mean) of the initial concentrations taken at time zero were defined as chemically compatible with regard to the drug substance tobramycin.

Physical Compatibility

Physical properties of the mixtures and of the mixture components were determined 1–2 hr after mixing and after 8–9 hr of storage. Values of pH were measured with pH test strips pH 4–7 and osmolality was determined via the freezing depression method with an osmometer (Osmomat-030, Gonotec GmbH, Berlin, Germany). Test suspensions were visually inspected with the unaided eye for any changes over the entire test period. Test solutions with no change in pH value, osmolality or visual appearance were defined as compatible and physically stable.

RESULTS

According to the conventional definition of compatibility, mixtures of inhalation solutions can be designated as physicochemical compatible, when stability (decomposition 10% or less) of each active ingredient and unchanged pH values, osmolality and physical appearance are proven over the entire test period (24 hr or less). Corresponding to this definition, mixtures of Pulmozyme® with GERNEBCIN® or TOBI® are to be designated as incompatible.

Dornase alfa activity decreased in each type of mixture tested (see Table 1, Figs. 1a,b and 2). In mixtures of Pulmozyme® with TOBI® (Fig. 1b) a significant loss of dornase alfa activity was obvious after 24 hr of storage. Mixtures of Pulmozyme® with GERNEBCIN® (Fig. 1a) exhibited more than 15% loss of enzymatic activity immediately after mixing. Most extensive loss was seen in mixtures of Pulmozyme® with pure solutions of sodium metabisulfite (Fig. 2), which is used as antioxidative excipient in GERNEBCIN®, but not in TOBI®. Sodium metabisulfite produces in aqueous solutions sulfurous acid, which acts as reducing agent. A major loss of dornase alfa activity was obvious after 1–2 hr of storage. The decrease of activity increased over storage time and inactivation was nearly completed after 28–29 hr of storage. In contrast dornase alfa activity remained unchanged in the unmixed Pulmozyme® control solution up to 29 hr of storage.

Tobramycin concentrations were found to be stable over a period of 8 hr in the mixtures of both tobramycin containing brands with Pulmozyme® inhalation solution and in the unmixed GERNEBCIN® control solution (compare Table 2). The concentrations retained nearly 100% of the initial tobramycin concentrations after mixing and storage in glass containers at room temperature.

TABLE 1—Enzymatic Activity of Dornase Alfa in Mixtures of Pulmozyme® With TOBI®, GERNEBCIN®, or 0.05% Sodium Metabisulfite Solution (Excipient in GERNEBCIN®) Expressed as Percentage (%) ± rel. SD (%) of Nominal Activity When Stored Under Ambient Light Conditions at Room Temperature

	Enzymatic activity of dornase alfa ± rel. SD (%)							
		Actual						
Test solution	Nominal	Initially after mixing	After 1–2 hr	After 7 hr	After 20 hr	After 24 hr	After 28–29 hr	After 33–34 hr
Control solution								
Pulmozyme®[1]	100	n.d.	n.d.	97.08 ± 3.4	96.83 ± 8.1	87.33 ± 7.5	95.00 ± 5.1	n.d.
Test solution								
TOBI® + Pulmozyme®[2]	100	88.07 ± 31.1	98.30 ± 9.5*	95.04 ± 7.1	n.d.	77.46 ± 31.4	79.33 ± 9.2	77.71 ± 8.6
GERNBECIN® + Pulmozyme®[3]	100	60.88 ± 28.5**	76.42 ± 9.4	83.28 ± 11.8	n.d.	66.97 ± 10.1	71.81 ± 16.0	74.33 ± 9.8
0.05% Sodium metabisulfite solution + Pulmozyme®[4]	100	87.42 ± 3.6	59.25 ± 14.9	n.d.	48.75 ± 33.8	n.d.	10.42 ± 101.3	n.d.

n.d., not defined.
[1]Enzymatic activity expressed as mean (n = 3) of the mean of 4 determinations of one sample at a time of 3 test solutions.
[2]Enzymatic activity expressed as mean (n = 6, *n = 5) of the mean of 4 determinations of one sample at a time of 9 test solutions.
[3]Enzymatic activity expressed as mean (n = 9, **n = 8) of the mean of 4 determinations of one sample at a time of 12 test solutions.
[4]Enzymatic activity expressed as mean (n = 3) of the mean of 4 determinations of one sample at a time of 3 test solutions.

Measured variations of the concentrations fell within the range of the relative standard deviation of the method.

Results of pH and osmolality measurements are shown in Table 3. Each of the nebulizable drug products (Pulmozyme®, TOBI®, and GERNEBCIN®) and the mixtures exhibited a pH in the range of 5.5 to 6.5, which corresponds to the limits set for nebulizer solutions in the Ph. Eur. (pH 3–8.5).[12] Mixtures of Pulmozyme® with TOBI® exhibited no significant changes of the pH, but in mixtures of Pulmozyme® with GERNEBCIN® a decrease of pH was registered after 8–9 hr of storage. A change of pH was also seen in mixtures of Pulmozyme® with 0.05% sodium metabisulfite solution. The pH of the mixture declined from pH 5–5.5 (measured after 2 hr of storage) to pH 4.5–5 after 4 hr of storage and remained unchanged up to the maximum observation interval of 28 hr.

Mixing of the isotonic Pulmozyme® nebulizer solution with the hypotonic tobramycin containing nebulizer solutions TOBI® and GERNEBCIN® produced hypotonic inhalation mixtures (osmolality about 200 mosmol). No significant changes of osmolality were measured over the storage period of 8 hr.

Visible changes were detectable in each type of mixtures inspected. Changes became visible 1 hr after mixing. Particles increased in number and size depending on the storage period. In mixtures of Pulmozyme® with sodium metabisulfite solution particles seemed to have a brown color after 20 hr of storage. The odor of mixtures of Pulmozyme® with GERNEBCIN® or sodium metabisulfite solution also worsened in correlation to the storage period.

DISCUSSION

Compounding of test solutions was selected to observe normal inhalation practice. In order to determine the stability of the inhalation mixture components we used two different methods. Quantitative analysis of dornase alfa activity was done with a kinetic colorimetric activity assay. This biological assay is not as accurate as a chemical assay, but this method was an available one and directly indicates the remaining drug activity resulting from potential degradation reactions. Dornase alfa caused hydrolysis of DNA results in production of free methyl green and change of absorbance at 600 nm is determined in the colorimetric activity assay. For each assay run a non-linear calibration curve had to be performed. Calibration failures resulted in varying determination time intervals for the test solutions and in a reduced number of analyzed test solutions (“n”) compared to the prepared test solutions. The DNase activity measured for an identical sample decreases during the assay course probably due to the instability of the DNA-methyl green substrate solution. For example within the same assay for an

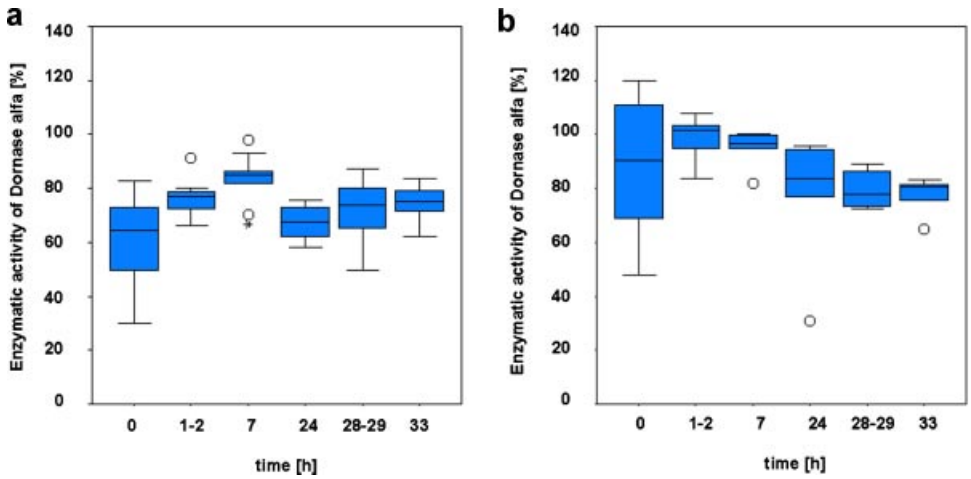

Fig. 1. a,b: Enzymatic activity of Dornase alfa in mixtures of Pulmozyme® with GERNEBCIN® 80 mg (a) or with TOBI® (b) expressed as percentage (%) of nominal activity when stored under ambient light conditions at room temperature.

unmixed sample with a nominal dornase alfa activity of 100% results varied between 99% at the beginning and 81% at the end of the run. Therefore we analyzed each sample once followed by a threefold repetition of the analysis in the identical sample order. The mean of four results for one sample was used for further interpretation. Results outside the calibration curve were ignored. While determining the precision of the chosen method this proceeding resulted in a mean dornase alfa activity of $89 \pm 8.3\%$ ($n = 15$) for nominal 100% and $78\% \pm 9.3\%$ for nominal 85% activity ($n = 39$). In part the relative standard deviation of 8.3% may also be caused by the 4,000-fold dilution in several steps during sample preparation. Due to the relative standard deviation of 8.3% a 10% loss of enzymatic activity can not be determined. As shown by Lichtinghagen the kinetic method can differentiate between 100% and 85% (i.e., 15% loss) of enzyme activity.[11] Because of the limited precision of the method we chose a study period of 2 days in order to get information about the course of inactivation. In clinical practice a test period longer than a few hours is not of relevance.

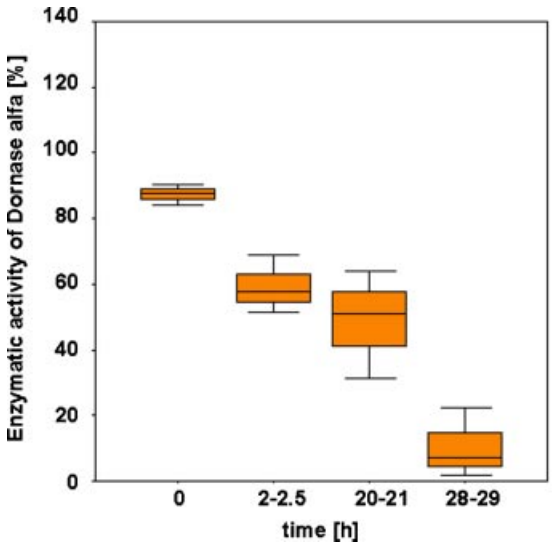

Fig. 2. Enzymatic Activity of Dornase alfa in mixtures of Pulmozyme® with 0.05% Sodium metabisulfite solution expressed as percentage (%) of nominal activity when stored under ambient light conditions at room temperature.

The test results confirm the importance of simulating clinical practice, when performing compatibility studies. The different drug products used contained different excipients which caused different compatibility results. Dornase alfa activity was more compromised in mixtures of Pulmozyme® with GERNEBCIN® 80 mg than in mixtures of Pulmozyme® with TOBI®, although the concentration of tobramycin in mixtures with TOBI® is higher (40 mg/ml) than in mixtures with GERNEBCIN® (17.8 mg/ml). As shown by the confirmatory experiments the enhanced decrease of activity is caused by the excipient sodium metabisulfite (see Table 1) used in 0.05% concentration in GERNEBCIN® (personal communication from the manufacturer). The difference in activity loss between mixtures with 0.05% sodium metabisulfite solution and mixtures with GERNEBCIN® might be explained by the different pH of the two test solutions. Mixtures of Pulmozyme® with 0.05% sodium metabisulfite solution are more acidic than mixtures of Pulmozyme® with GERNEBCIN®. As sodium metabisulfite degrades to sulfurous acid in acidic solutions[13] the higher extent of sulfurous acid nascent and/or the pH change itself might affect DNAse acitivity.[8] The observed pH and odor changes in mixtures of Pulmozyme® with GERNEBCIN® and 0.05% sodium metabisulfite solution

TABLE 2—Stability of Tobramycin in Mixtures of Pulmozyme® With TOBI® or GERNEBCIN® Stored Under Ambient Light Conditions at Room Temperature

	Tobramycin concentration (μg/ml) ± rel. SD (%)			Initial tobramycin concentration remaining ± rel. SD (%)[2]
		Actual		
Test solution	Nominal	Initially after mixing	After 8 hr	After 8 hr
Control solution				
GERNEBCIN® + 0.9% NaCl[1]	8	8.122 ± 6.11	7.977 ± 1.38	98.5 ± 6.7
Test solution				
TOBI® + Pulmozyme® [1]	8	8.401 ± 1.36	8.757 ± 2.07	104.2 ± 2.3
GERNEBCIN® + Pulmozyme® [1]	8	8.008 ± 1.61	7.728 ± 4.38	96.5 ± 3.4

[1]Concentrations expressed as mean (n = 6) of duplicate determinations of one sample of each of 3 test solutions.
[2]Drug concentrations in samples taken at time 0 designated as 100%.

correspond with the results of the colorimetric assay and the suggested explanation.

Tobramycin concentration remained unchanged in the test solutions which is assumed to correlate with unchanged antimicrobial activity, but was not studied explicitly.

In order to designate mixtures of Pulmozyme® with TOBI® as compatible, additional assays for determination of dornase alfa integrity, for example, an ion exchange chromatography assay or reversed-phase HPLC are required. Even if efficacy is shown to be comparable, a difference in the safety profile should be excluded. Aggregates of protein drug substances more likely induce immunogenicity than the monomers and thereby decrease clinical safety. For safety reasons formation of sub-visible dornase alfa aggregates should be studied, for example, by size exclusion chromatography. In addition the nebulization properties of the proposed inhalation mixture are to be studied before a final recommendation of simultaneous nebulization can be made.[14] The process of aerosol production also might influence compatibility of different drug solutions (physical and mechanical forces, temperature drop during nebulization).[15] Moreover simultaneous nebulization of nebulizable medications can affect drug delivery of the components by for example altering the aerosolized particle size distribution or the total mass output and inhaled mass.[15–17] Values of pH and osmolality are important factors influencing the tolerability of nebulizable drugs. Inhalation of acidic and/or hypotonic drug formulations may induce bronchoconstriction and cough.[18–20] Nebulized drug formulations with a pH in the lower range of the established Ph. Eur. limits and osmolarities lower than 150 mOsm/L (no limits defined in the Ph. Eur.) may cause intolerance reactions. Mixing Pulmozyme® with tobramycin nebulizer solutions advantageously increases the osmolality of the hypotonic tobramycin nebulizer solutions (see Table 3). The importance of osmolality and pH of aerosolized drugs in provoking bronchoconstriction is shown in asthmatic patients, but it is not yet studied whether these parameters also provoke bronchoconstriction in patients with cystic fibrosis. Probably some cystic fibrosis patients are affected because approximately half of them have a hyperreagibile bronchial system.[21] Mixing drug products generally decreases concentrations of active ingredients and excipients. By this the bronchoconstrictive effects of excipients like sodium metabisulfite are also diminished.[18] Decreased concentrations of preservatives may also lead to reduced microbiological stability of the mixtures. Therefore mixtures can only be prepared directly before nebulization and sureplus quantities should not be stored.

The clinical relevance of the inhalation sequence of the different drugs used and outcome differences according to

TABLE 3—Osmolality and pH Values of Pulmozyme®, TOBI®, and GERNEBCIN® (Un)Mixed Nebulizer Solution Stored Under Ambient Light Conditions at Room Temperature

Nebulizer solution	pH	1–2 hr after mixing	8–9 hr after mixing	Osmolality (osmol/kg) ± rel. SD (%)	1–2 hr after mixing	8–9 hr after mixing
Pulmozyme®	5.5–6	n.a.	n.a.	0.276 ± 0.27 (n = 8)	n.a.	n.a.
TOBI®	6.1–6.5	n.a.	n.a.	0.167 ± 0.59 (n = 8)	n.a.	n.a.
GERNEBCIN®	5.5–6.1	n.a.	n.a.	0.096 ± 0.54 (n = 8)	n.a.	n.a.
Pulmozyme® + TOBI®	n.a.	6.1	6.1	n.a.	0.214 ± 0.71 (n = 5)	0.214 ± 0.21 (n = 5)
Pulmozyme® + GERNEBCIN®	n.a.	5.5	5.0	n.a.	0.198 ± 0.58 (n = 5)	0.199 ± 0.91 (n = 5)

n.a., not appropriate.

consecutive or simultaneous inhalation are not investigated by clinical studies. Sometimes patients are informed to inhale Pulmozyme® before and tobramycin after their physiotherapy session.[22] However in an international survey at multiple CF-Centres, Borsje et al.[23] found that Pulmozyme® is nebulized as often before as after physiotherapy. Van der Giessen showed that inhalation of Pulmozyme® immediately after physiotherapy is equally or even more effective as inhalation 30 min before physiotherapy.[24] As long as clinical studies on the matter are missing, simultaneous inhalation of Pulmozyme® and TOBI® could be an option for CF patients, at least from a clinical and practical point of view, and further investigations on compatibility are worthwile. Provided that physico-chemical stability of dornase alfa and Tobi® drug admixtures is unequivocally demonstrated, clinical studies comparing consecutive and simultaneous inhalation are to be initiated.

CONCLUSIONS

Experimental studies simulating the mixture of drug products containing dornase alfa and tobramycin for inhalation yielded different results depending on the formulation of the drug product and the components. Tobramycin concentration was not affected by mixing the drug products. Dornase alfa activity is especially affected by sodium metabisulfite, used as excipient in GERNEBCIN®. Patients should be advised, not to mix Pulmozyme® with GERNEBCIN® because of the incompatibility reaction. In order to define efficacy and safety of mixtures of Pulmozyme® with TOBI® further studies are needed which clearly indicate the integrity and activity of dornase alfa and tobramycin. In addition the nebulization properties of this mixture and ultimately clinical efficacy are to be studied before a final recommendation of simultaneous nebulization can be made.

Materials

Pulmozyme® nebulizer solution 2,500 U/ 2.5 ml: Roche Pharma AG, Grenzach-Whylen, Germany, lot: L00130, L00121, L00115, and L00144; GERNEBCIN® 80 mg/ 2 ml: Infectopharm, Heppenheim, Germany, lot: G060301 and G070407; TOBI® 300 mg/5 ml: Chiron, München, Germany, lot: 04K2C, 02K4B, and 05K3C; sodium metabisulfite dilution: catalog number 2467, Caelo, Hilden, Germany, lot: 32274304; sodium chloride 0.9%, preservative free: Braun, Melsungen, Germany, lot: 4411C12; commercial fluorescence immunoassay buffer X-Systems Verdünnungspuffer: Abbott, lot: 28292M102); DNA, catalog number 223646, Roche Diagnostics; Methyl green, catalog number M884, Sigma, Steinheim, Germany; Hepes, catalog number H-3375, Sigma; EDTA (Titriplex III), catalog number 8418, Merck, Darmstadt, Germany; Acetat-NaOH, catalog number A1045, Applichem, Darmstadt, Germany; $CaCl_2$, catalog number 2381, Merck; $MgCl_2 \times 6\ H_2O$, catalog number 5833, Merck; Bovines Serum Albumin (BSA), Albumin Fraktion V, catalog number 735078, Roche Diagnostics; Thiomersal, catalog number T-8784, Sigma; Tween® 20, catalog number P-1379, Sigma; H_2O_2 35%: catalog number 1.08600.1000, Merck, lot: K34259500 505; Spezialindikator pH 4.0–7.0, 0.3 increments: catalog number 109542, VWR International GmbH, Darmstadt, Germany; Acilit® pH 0–6.0, 0.5 increments: catalog number 109531, VWR International GmbH; plastic containers: catalog number 55.468, Sarstedt, Nümbrecht, Germany; glass containers: catalog number Lenz 3.0214.13, VWR International GmbH.

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