ELSEVIER

International Journal of Pharmaceutics 189 (1999) 215–225

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Inhalation of tobramycin in cystic fibrosis Part 2: Optimization of the tobramycin solution for a jet and an ultrasonic nebulizer

P.P.H. Le Brun [a,*], A.H. de Boer [b], D. Gjaltema [b], P. Hagedoorn [b], H.G.M. Heijerman [c], H.W. Frijlink [b]

[a] *Central Hospital Pharmacy The Hague, P.O. Box 43100, 2504 AC The Hague, The Netherlands*
[b] *Department of Pharmaceutical Technology and Biopharmacy, University of Groningen, Groningen, The Netherlands*
[c] *Adult Cystic Fibrosis Center, Department of Pulmonology, Leyenburg Hospital, The Hague, The Netherlands*

Received 27 April 1999; received in revised form 12 July 1999; accepted 19 July 1999

Abstract

The inhalation of tobramycin is part of current cystic fibrosis (CF) therapy. Local therapy with inhaled antibiotics has demonstrated improvements in pulmonary function. Current inhalation therapy is limited by the available drug formulations in combination with the nebulization time. The aim of this study is to develop a highly concentrated tobramycin solution for inhalation. Several tobramycin solutions, ranging from 5 to 30% (m/v), were compared after aerosolation with a jet and with an ultrasonic nebulizer. Laser diffraction and cascade impactor analysis were used for characterization of the aerosolized solutions. The output rate was determined in volume and mass output per minute. From the output rate measurements, it was concluded that a 20% tobramycin solution is the optimal and maximal concentration to be aerosolized. The jet nebulizer was most suitable. Using the jet nebulizer and the 20% solution, it is possible to administer a dosage of 1000 mg tobramycin by inhalation within 30 min. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Cystic fibrosis; Tobramycin; Inhalation; Nebulizer; Laser diffraction; Cascade impactor

1. Introduction

The inhalation of tobramycin is part of current cystic fibrosis (CF) therapy. Tobramycin and other inhaled antibiotics are used to delay or prevent chronic infection with *Pseudomonas aeruginosa* and to prevent clinical detoriation in patients chronically colonized. Both controlled and uncontrolled studies have demonstrated improvements in pulmonary functions after inhalation of tobramycin (Touw et al., 1995; Davis et al., 1996; Mukhopadhyay et al., 1996; Webb and Dodd, 1997; Ramsey et al., 1999). Recently a tobramycin solution for inhalation (TOBI®), indicated for the management of CF patients with

* Corresponding author. Tel.: +31-70-321-7217; fax: +31-70-321-7110.

PII: S0378-5173(99)00252-5

Pseudomonas aeroginosa infections, was introduced in the US.

The aim of antibiotic inhalation therapy is to deliver high amounts of drug to the sites of infection. However, it is not possible to reach all sites with local therapy, making additional intravenous therapy a requirement for adequate treatment of infectious exacerbations. In practice, it would be more convenient for patients to receive a therapy of high local amounts of antibiotics which also leads to an adequate serum concentration. Based on knowledge of intravenous therapy, peak serum concentrations of 6–12 mg/l are required for optimal treatment of exacerbations. Theoretically, these peak concentrations can be attained by inhalation of a high dose of tobramycin (Touw et al., 1997). On the other hand, it is not known whether these high plasma concentrations are required to treat exacerbations when the antibiotic is provided by inhalation.

Ramsey et al. (1999) measured a mean value of approximately 1 mg/l 1 h after a dosage of 300 mg tobramycin by inhalation. Serum concentrations were measured on day 3 and day 10 during a treatment period of 28 days. In a study by Touw et al. (1997) mean tobramycin peak concentrations of 1.27 mg/l (range 0.19–2.57) were measured after inhalation of 600 mg. Extrapolation of the pharmacokinetic data to a three times daily inhalation of 600 mg resulted in a steady state serum concentration of 1.5 mg/l (range 0.2–4.7 mg/l). In this study an ultrasonic nebulizer was used.

It can be calculated that a dosage of at least 2 g has to be inhaled in order to reach therapeutic serum concentrations. Commercial solutions of tobramycin for inhalation contain concentrations of 40–60 mg/ml. This means that high amounts of tobramycin cannot be administered with current available formulations for inhalation unless extreme inhalation times are accepted.

The physical chracteristics of drug solutions have an effect on output rate, particle size distribution and possible irritation after inhalation (McCallion et al., 1995, 1996a). Therefore, an appropriate nebulization system needs to be assessed for every drug solution. Moreover, drug deposition data should be available (Hurley et al., 1994; McCallion et al., 1996b; Coates et al., 1997; Weber et al., 1997).

Recently the in vitro characteristics of a 10% (100 mg/ml) tobramycin solution have been investigated (Le Brun et al., 1999a). Using this formulation, administration of 20 ml is required to administer 2 g of tobramycin. The time required to inhale such an amount is not acceptable for most patients. Therefore the application of a new, highly concentrated, tobramycin formulation has been investigated.

In this paper the development and the characteristics of the new tobramycin formulation are described. Particle size distributions of the aerosol clouds were measured with laser diffraction technique. Deposition data were obtained from cascade impactor analysis.

In the above mentioned previous study on a formulation of 10% tobramycin, the characteristics of 14 nebulizers were compared. It was concluded that three jet nebulizers are most suitable for the inhalation of a 10% tobramycin solution. One of these nebulizers, the Porta-Neb Ventstream, was selected for further investigations and used in the current study on different tobramycin formulations.

Inhalation of tobramycin is also possible with an ultrasonic nebulizer. Although the characteristics of an ultrasonic nebulizer are less suitable than those of a jet nebulizer to administer a 10% solution of tobramycin, it is not certain whether this conclusion is also valid for other formulations of tobramycin. Therefore, one of the better ultrasonic devices, the Medix Sonic 2000, was used in the current study as a representative of the ultrasonic nebulizers.

2. Materials and methods

2.1. Tobramycin solution

Tobramycin sulphate (USP XXIII quality, Pharmacin, Zwijndrecht, The Netherlands) is soluble in water. Solutions of 5, 10, 20 and 30% (m/v) were prepared in water for injection.

The osmolality was measured using a freezing-point-depression osmometer (Osmometer Knauer

4050, Wilton, Etten Leur, The Netherlands). The shear viscosity was determined using a rotation viscometer (Brookfield Engineering Laboratories, van Oortmerssen, Den Haag, The Netherlands). The viscosity was determined at a rotation speed of 250 rpm. The density was measured by weighing a volume of 10.00 ml. Furthermore, the pH (Radiometer, Copenhagen, Denmark) was determined. All physical measurements were performed at ambient temperature.

2.2. Devices

The Porta-Neb Ventstream® (Medic Aid, Romedic, Meersen, The Netherlands) was used as a representative of the jet nebulizers.

The performance of this nebulizer was compared to an ultrasonic device, the Medix Sonic 2000® (Huisman, Tiel, The Netherlands).

2.3. Particle size distribution

The particle size distribution of the aerosol cloud from the nebulizers was measured with a Sympatec HELOS Compact laser diffraction analyzer, model KA (Sympatec GmbH, Clausthal-Zellerfeld, Germany). A lens with 100 mm focal-length for the size range 0.9–175 mm was used for all experiments. Fraunhofer theory was applied for the calculation of the droplet size distribution from the laser diffraction pattern. In order to standardize laser diffraction analysis, a special adapter was developed. This adapter was fixed to the support in the measuring zone of the Sympatec and connected to a vacuum system, including a Venturimeter for flow measurement, a single stage liquid impinger for the separation of droplets from the air stream and a flow controller. The adapter could be tilted and had an exchangeable front in order to accept both nebulizers used in the study, having different lengths, angles and shapes of their mouthpieces. The distance between the mouthpieces and the laser beam was adjusted to 6.5 cm; the (inspiratory) flow rate through the adapter was adjusted to 20–40 l/min.

The cups of the nebulizers were filled with 4.5 ml for the Porta-Neb Ventstream and with 4.0 ml solution for the Medix Sonic 2000, respectively. The tobramycin concentration ranged from 0 to 30%.

Droplet size distribution was not measured continuously, but 10 s, 1.5; 3; 4.5; 6; 9 and 12 min after the start of nebulization. Measuring time at each interval was 10 s. Both nebulizers were measured in triplicate according to these procedures.

The mean results from all laser diffraction analysis are expressed as X_{10}, X_{50} and X_{90}-values, being the diameters corresponding with respectively 10, 50 and 90% of total volume from cumulative percentage undersize curves

All measurements were performed at ambient temperature.

2.4. Nebulizer output

The output of nebulizers can be described by the aerosolized volume or the aerosolized mass of drug. The nebulized volume can be determined simply by weighing the nebulizer before and after use. Results may be misleading because they do not take into account the increase in drug concentration within the nebulizer caused by evaporation of the solvent. Therefore drug output rate in mg/min is a better parameter for the nebulizer output.

After 6 min of nebulization, the nebulizer was weighed and the nebulized volume was calculated.

The tobramycin concentration of the solution before nebulization and of the residual volume in the nebulizer after nebulization was measured. The nebulized output rate was expressed in ml/min and in mg/min.

All measurements were performed at ambient temperature.

2.5. Tobramycin assay

The tobramyin concentration was determined using fluorescence polarization immunoassay (AxSYM, Abbot). The error of this method is less than 5%. Every sample was diluted 25 000 times to a concentration of approximately 4 mg/ml and measured twice. The concentration in the samples was calculated from the results of a standard solution of 10% tobramycin measured in the same run.

2.6. Cascade impactor analysis

For cascade impactor analysis a four stage glass constructed Lenz Labor impactor was used. All stages were wetted with distilled water. Particles of decreasing sizes are deposited on the subsequent stages. The fractions deposited on stages 3 and 4 are assumed to represent the respirable part of the inhaled dose. The theoretical cut-off diameters of the impactor at different flow rates and for different concentrations of tobramycin are presented in Table 1.

The nebulizers were connected to the impactor. All nebulization experiments were continued for 6 min.

First the droplet size distribution of a 10% solution was analyzed in duplo at three different flow rates of 15, 20 and 30 l/min.

Subsequently, the droplet size distribution of a 20 and a 30% solution was measured in duplo at a flow rate of 20 l/min.

The distributions from the Porta-Neb Ventstream were compared to those from the Medix Sonic 2000 at a flow rate of 20 l/min and with solutions of 10, 20 and 30% tobramycin.

Since the objective of this study was to characterize a high dose tobramycin formulation, the 5% solution was not evaluated by cascade analysis.

All measurements were performed at ambient temperature.

3. Results

3.1. Tobramycin solution

The characteristics of the several tobramycin solutions are presented in Table 2.

The osmolality, density and viscosity increased with the concentration. The pH for all solutions was within the range of 7–8.

3.2. Particle size distribution

The particle size distributions of the Porta-Neb Ventstream and the Medix Sonic 2000 are presented as X_{10}, X_{50} and X_{90}-values from cumulative undersize curves in Figs. 1 and 2, respectively. The values are a mean of all measuring times for all three series of measurements. The (inhalation) flow rates were 20 and 40 l/min in these experi-

Table 1
Theoretical cut-off diameters in micrometers of the stages of the cascade impactor[a]

Flow rate (l/min)	Concentration (% w/w)	Stage 1	Stage 2	Stage 3
15	10	40.6	20.8	10.3
20	10	35.2	18.0	8.9
20	20	34.4	17.6	8.7
20	30	33.2	17.0	8.4
30	10	28.7	14.7	7.3
40	10	24.9	12.7	6.3

[a] The diameters are calculated at different flow rates and for different tobramycin concentrations.

Table 2
Characteristics of the tobramycin solutions

Concentration (%)	pH	Viscosity (mPa/s)	Density (g/ml)	Osmolality (mmol/kg)
5	7.4	2.3	1.02	109
10	7.5	2.6	1.06	230
20	7.6	3.2	1.11	483
30	7.8	6.7	1.19	1044

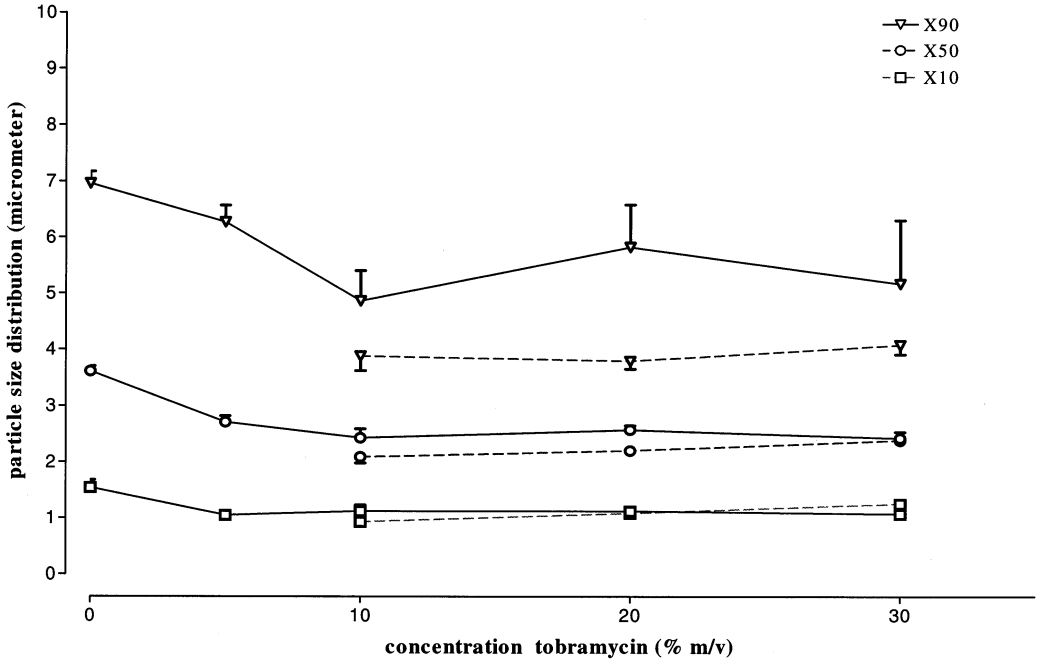

Fig. 1. A representation of the droplet size distribution of the Porta-Neb Venstream at several concentrations of tobramycin and at flow rates of 20 l/min (straight lines) and of 40 l/min (dotted lines), respectively. The droplet size distribution is expressed as X_{10}, X_{50} and X_{90} values $\pm$ S.D. from cumulative percentage undersize curves.

ments. For the Porta-Neb Ventstream, the particle size distribution was not significantly different in the concentration range from 5 to 30% tobramycin. For the Medix Sonic 2000, the particle size distribution decreased when the concentration increased from 5 to 10%. At concentrations of 10 and 20% the particle size was not significantly different. Furthermore, it was not possible to aerosolize a 30% tobramycin solution with the Medix Sonic 2000. Reasons for this might be the high viscosity and the higher density of the solution.

Furthermore, it was not possible to aerosolize distilled water with the Port-Neb Ventstream at a flow of 40 l/min.

It can be concluded that the particle size distribution of the aerosols generated by the Porta-Neb Ventstream is hardly influenced by the tobramycin concentration up to 30% m/v.

3.3. Nebulizer output

The ouput rates in mg/min and in ml/min of the Porta-Neb Ventstream and the Medix Sonic 2000 are presented in the Fig. 3. The output rate was measured at a flow rate of 20 l/min. The output rate of both nebulizers can be compared in this figure. As expected the output rate of the Porta-Neb Ventstream is higher.

In the concentration range of 5–20% the tobramycin output increases with the concentration. However, between 20 and 30% no further increase is seen. Apparently the higher tobramycin concentration in the droplets is compensated by the reduced formation of droplets.

Especially the rapid decrease of the volume output rate of the Medix Sonic at higher concentrations is remarkable. This decrease also explains the fact that a higher tobramycin concentration does not result in higher output rates of tobramycin.

The output rate of the Porta-Neb Ventstream was also measured at 40 l/min. The output rate at 40 l/min is compared to the rate at 20 l/min in Fig. 4. The output rate is higher at a higher flow. With the Porta-Neb Ventstream there seems to be an optimum for the output rate and the concentration of the solution to be nebulized. The output rate in mg/min is maximized at a concentration of 20% of tobramycin at both flow rates.

3.4. Cascade impactor analysis

The distribution of a 10% tobramycin solution at three different flows for the Porta-Neb Ventstream is presented in Fig. 5. The deposition pattern is comparable at flow rates of 15 and 20 l/min. The deposition is somewhat higher at a flow rate of 30 l/min. The mean over all output is 24.5, 29.9 and 31.9 mg/min at a flow of 15, 20 and 30 l/min, respectively.

In Fig. 6 the deposition patterns are presented for the Porta-Neb Ventstream at a flow rate of 20 l/min for three different concentrations of 10, 20 and 30% tobramycin. The mean over all output is 29.9, 58.0, and 51.6 mg/min for the tobramycin solutions of 10, 20 and 30%, respectively.

The depostion pattern of the Porta-Neb Ventstream and the Medix Sonic 2000 were compared at a flow rate of 20 l/min and with two solutions of tobramycin (10 and 20%). It was not possible to aerosolize higher concentrations with the Medix sonic 2000. The data are presented in Fig. 7. The mean over all output of the Medix sonic 2000 is 19.0 and 13.4 mg/min for the tobramycin solutions of 10 and 20%, respectively, at a flow of 20 l/min.

4. Discussion

Usually, the concentration of aqueous tobramycin solutions for aerosolation ranges from 4 to 6%. The experiments have shown that it is possible to aerosolize higher concentrations of tobramycin.

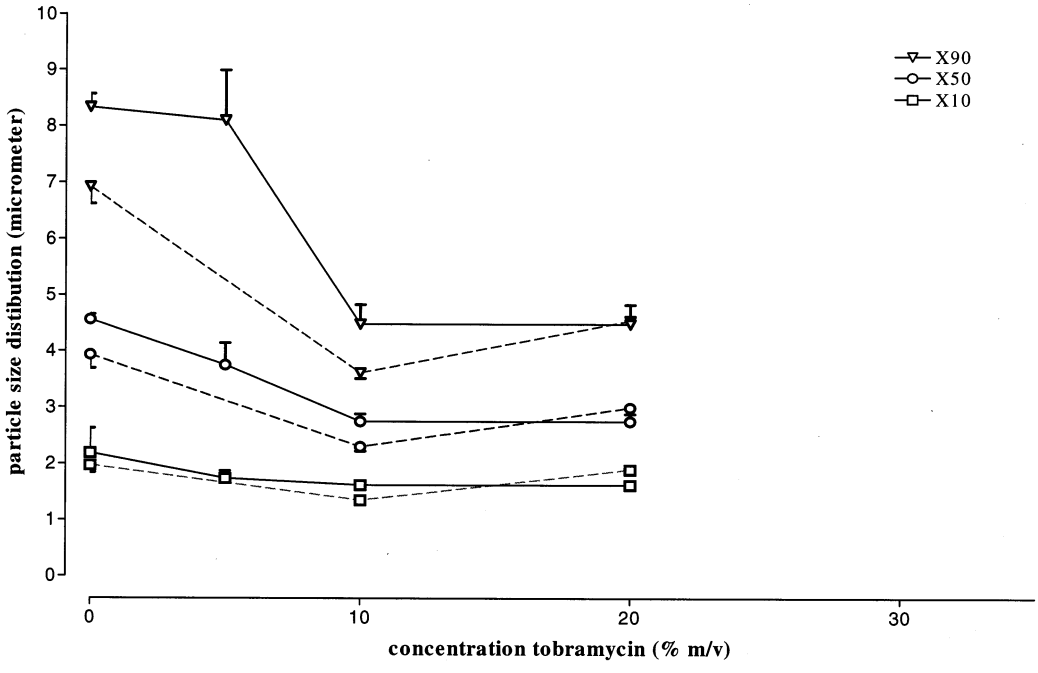

Fig. 2. A representation of the droplet size distribution of the Medix Sonic 2000 at several concentrations of tobramycin and at flow rates of 20 l/min (straight lines) and of 40 l/min (dotted lines), respectively. The droplet size distribution is expressed as X_{10}, X_{50} and X_{90} values $\pm$ S.D. from cumulative percentage undersize curves.

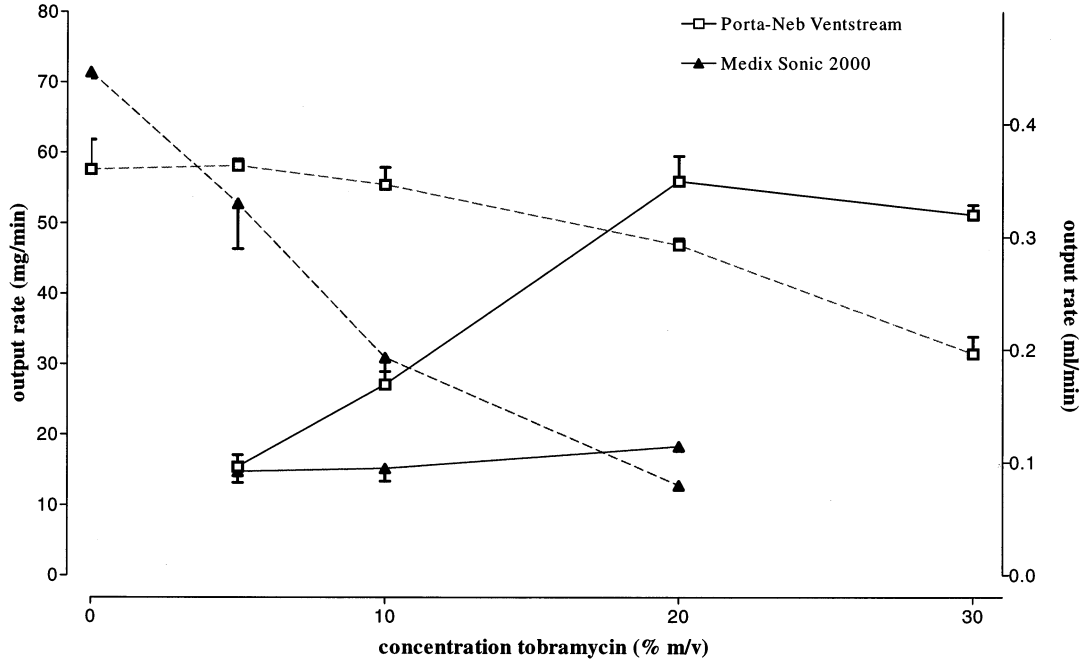

Fig. 3. A representation of the mean output rate in mg/min (straight lines) and in ml/min (dotted lines) ± S.D. from the Porta-Neb Ventstream and the Medix Sonic 2000 for several concentrations of tobramycin at a flow rate of 20 l/min.

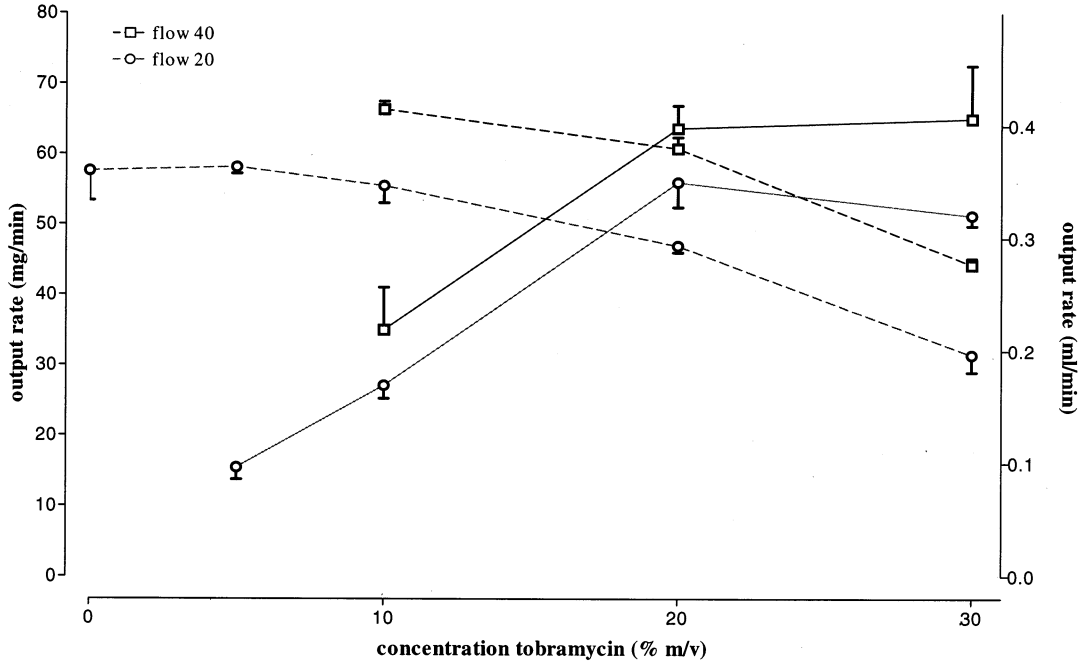

Fig. 4. A representation of the mean output rate in mg/min (straight lines) and in ml/min (dotted lines) ± S.D. from the Porta-Neb Ventstream for several concentrations of tobramycin at flow rates of 20 and 40 l/min, respectively.

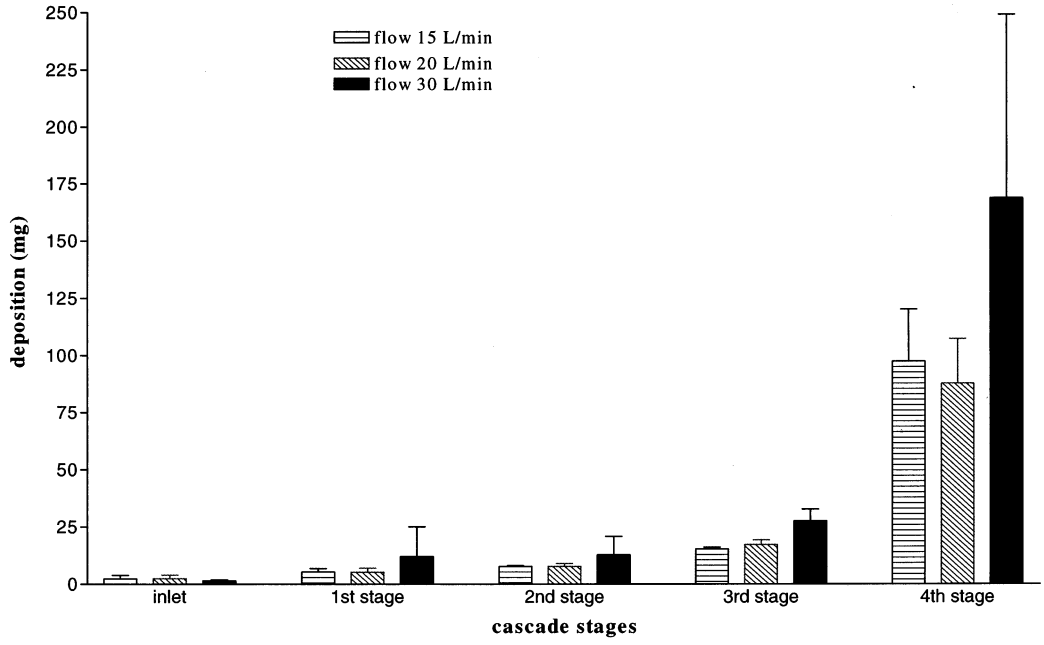

Fig. 5. A representation of the absolute deposition of tobramycin in the respective stages of the cascade impactor after aerosolation with the Porta-Neb Ventstream at different flow rates.

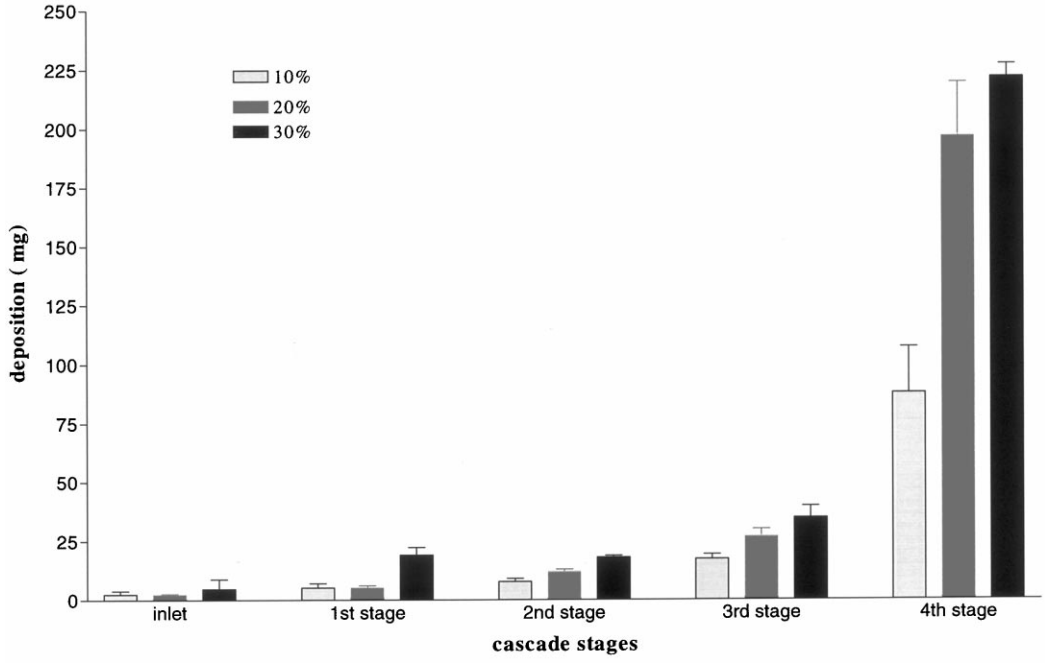

Fig. 6. A representation of the absolute deposition of tobramycin in the respective stages of the cascade impactor after aerosolation of different concentrations with the Porta-Neb Ventstream at a flow rate of 20 l/min.

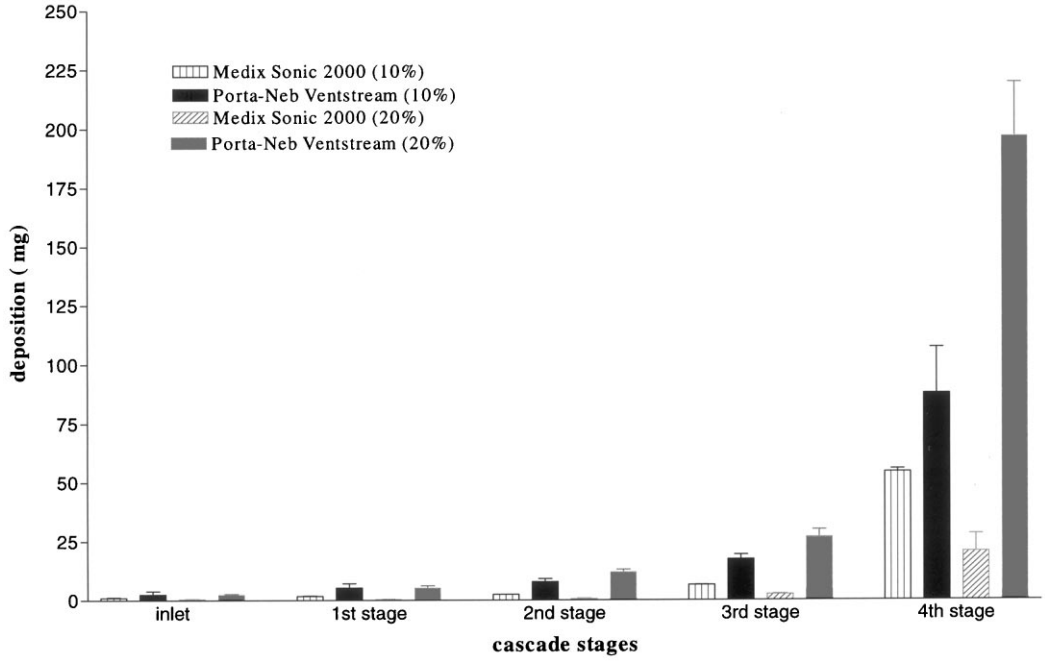

Fig. 7. A representation of the absolute deposition of tobramycin in the respective stages of the cascade impactor after aerosolation of a 10 and a 20% solution, respectively, with the Porta-Neb Ventstream and the Medix Sonic 2000 at a flow rate of 20 l/min.

After comparing several concentrations of tobramycin, ranging from 5 to 30%, an optimum was found for the mass output rate at a concentration of 20% using the jet nebulizer. In contrast, the results with the ultrasonic nebulizer showed no improvements at higher concentrations. The optimal output rate of the ultrasonic nebulizer was found to be at a 10% tobramycin concentration.

The aerosolation of concentrations above the optimum did not result in higher mass output rates. This can be explained by the increasing density and viscosity of the solutions. In a jet nebulizer the gas flow from the compressor passes a very narrow hole, known as a venturi. A tube or feeding system surrounding the gas flow tube contains the drug solution. After passing the narrow hole, the gas flow transfers its momentum to the liquid surrounding the venturi. The liquid is drawn out into fine ligaments that collapse into droplets under influence of surface tension. The higher the density and viscosity, the more difficult it is to form droplets and the lesser the output rate from the nebulizer.

In an ultrasonic nebulizer, droplets are formed by ultrasonic vibrations. At a given frequency the threshold for the forming of droplets is determined by the density and the viscosity of the liquid (O'Callaghan and Barry, 1997). The higher the viscosity, the more energy is required to form droplets, resulting in a lower output from the nebulizer.

A decreasing volume output rate was observed at higher concentrations. As long as enough volume could be aerosolized, increasing concentrations of the solution cause a higher mass output rate. However, at a certain concentration the nebulizer cannot generate enough volume output and the mass ouput will reach the optimum. For the jet nebulizer this optimum was found at a concentration of approximately 20% tobramycin.

The same observations were made with the ultrasonic nebulizer. However, the driving force for aerosolation of the tobramycin solutions was

demonstrated to be less since the optimum output was already found at a 10% concentration.

The particle size distribution is another important factor determining the efficacy of a nebulizer.

In general, droplets with a diameter of 1–5 mm are assumed to be the respirable fraction of an aerosol. This fraction may be deposited in the large and small airways and the alveoli by impaction and sedimentation. Droplets larger than 5 mm will impact in the upper airways, whereas particles smaller than 1 mm are known to be partly exhaled again (Newman and Clarke, 1983).

For the Porta-Neb Ventstream we observed that the diameter of almost 90% of the aerosolized volume is within the desired range for a tobramycin concentration of 10–30%.

The particle size distribution was measured by a laser diffraction analyzer at simulated (inhalation) flows of 20 and 40 l/min.

On average, with a breathing pattern of 20 breathings/min and a tidal volume of 500–1000 ml, the inhalation flow of a patient rates between 10 and 20 l/min. Higher flow rates are also possible during inhalation. Therefore, a low flow rate (20 l/min) and a high flow rate (40 l/min) has been chosen in the experiments.

An acceptable particle volume size was measured at both flow rates and, furthermore, a higher output rate at a higher (inhalation) flow. In both situations the droplet sizes are in the respirable range.

In practice, a different flow rate might result in a different effect. From deposition studies with radiolabeled aerosols it is known, that a higher flow rate results in a higher central lung deposition, whereas a lower flow rate results in a more peripheral deposition (Laube, 1998). This also means that, in principle, drug targetting is possible. Up until now, it is not clear of this phenomena is relevant for the treatment of infections with inhaled antibiotics in CF patients.

The results of the laser diffraction analyses were confirmed by the measurements with cascade impactor. For a 10% solution of tobramycin, a higher (inhalation) flow results in a somewhat higher deposition (Fig. 5).

Deposition measurement with the higher concentrations were performed at a flow rate of 20 l/min. As before, in these experiments a further increase in concentration of 20–30% did not result in a significant increase of the deposition of tobramycin on the third and fourth stage of the impactor. Again, an optimum concentration for the Porta-Neb Ventstream was found at approximately 20%. The jet nebulizer performed better than the ultrasonic for the tobramycin solutions.

The mass output rate of the 20% tobramycin solution in the cascade impactor was comparable to the output rate in the laser diffaction experiments.

Both methods, laser diffraction and cascade impactor, proved to be suitable for nebulizer efficacy measurements.

The osmolality of a 20% solution is 483 mmol/kg. This value will be tolerated after inhalation, since an osmolality of an antibiotic solution in the range of 150–550 mmol/kg is acceptable for inhalation formulations (Weber et al., 1997). The pH of the 20% tobramycin solution was 7.6, an acceptable value for aerosolized solutions.

As expected, the viscosity increases with the concentration. A small increase was observed in the range from 5 to 20% tobramycin. The viscosity doubles when the concentration increases from 20 to 30%. The increase in viscosity correlated with a decrease in output rate. The output of the ultrasonic nebulizer was more sensitive to an increase in the viscosity than the jet nebulizer.

It was intended to treat exacerbations with inhalation therapy. In practice, a maximum nebulization time of 30 min is acceptable for patients. An optimum output rate of almost 60 mg/min was found (Fig. 6). Considering inhalation and exhalation time, this means that a maximum amount of 900–1000 mg tobramycin can be administered within 30 min using a 20% solution at an inhalation flow of 15–20 l/min.

In a patient study, a maximum tobramycin concentration up to 3.7 mg/l in plasma after inhalation of 600 mg was found (Le Brun et al., 1999b). This means that plasma concentrations of 5–6 mg/l can be reached with inhalation therapy using the 20% solution. Compared to intravenous therapy these values are low. Plasma tobramycin levels at least 8 mg/l are necessary to treat exacerbations with intravenous administration. These

high levels are necessary to reach the target area in the lung. However, after inhalation of tobramycin, most of the target area is reached directly. To reach all infectious sites, a sufficient tobramycin plasma concentration is also required. To date, it is not known which plasma levels are necessary for adequate treatment when aerosolized administration of tobramycin is used only.

Up until now, exacerbations could not be treated by inhalation therapy of antibiotics alone. It has been found that a higher amount of tobramycin than used until now can be administered. It is, therefore, worthwhile to investigate if the maximum possible amount of aerosolized tobramycin is useful for treatment of exacerbations.

5. Conclusions

It is concluded that a 20% tobramycin solution is the optimal concentration for high dose administration with the jet nebulizer and the ultrasonic nebulizer. The highest output rate is found for the jet nebulizer. It is not possible to nebulize high tobramycin concentrations ($>20\%$ w/v) with the ultrasonic nebulizer.

For both nebulizers it was observed that the diameter of almost 90% of the aerosolized volume is within the desired size range of 1–5 mm.

For high dose administration of tobramycin, the jet nebulizer seems the best choice.

References

Coates, A.L., MacNeish, C.F., Meisner, D., Keleman, S., Thibert, R., MacDonald, J., Vadas, E., 1997. The choice of jet nebulizer, nebulizing flow and addition of albuterol affects the output of tobramycin aerosols. Chest 111, 1206–1212.

Davis, P.B., Drumm, M., Konstan, M.W., 1996. Cystic fibrosis. Am. J. Respir. Crit Care Med. 154, 1229–1256.

Hurley, P.K., Smye, S.W., Cunliffe, H., 1994. Assessment of antibiotic aerosol generation using commercial jet nebulizers. J. Aerosol Med. 7, 217–228.

Laube, B.L., 1998. Measurement of aerosol deposition in CF. Pediatr. Pulm. 21 (Suppl 17), 181–182.

Le Brun, P.P.H., De Boer, A.H., Gjaltema, D., Hagedoorn, P., Heijerman, H.G.M., Frijlink, H.W., 1999a. Inhalation of tobramycin in cystic fibrosis. Part 1: The choice of a nebulizer. Int. J. Pharm., in press.

Le Brun, P.P.H., Vinks, A.A.T.M.M. Vinks, Touw, D.J., Hekelaar, N., Mannes, G.P.M., Brimicombe, W., Frijlink, H.W., Heijerman, H.G.M., 1999b. Improvement of tobramycin inhalation with a jet nebulizer; a pharmacokinetic analysis. Ther. Drug Mon., in press.

McCallion, O.N.M., Taylor, K.M.G., Bridges, P.A., Thomas, M., Taylor, A.J., 1995. Nebulization of fluids of different physicochemical properties with air-jet and ultrasonic nebulizers. Pharm. Res. 12, 1682–1688.

McCallion, O.N.M., Taylor, K.M.G., Thomas, M., Taylor, A.J., 1996a. The influence of surface tension on aerosols produced by medical nebulisers. Int. J. Pharm. 129, 123–136.

McCallion, O.N.M., Taylor, K.M.G., Bridges, P.A., Thomas, M., Taylor, A.J., 1996b. Jet nebulizers for pulmonary delivery. Int. J. Pharm. 130, 1–11.

Mukhopadhyay, S., Singh, M., Cater, J.I., Ogston, S., Franklin, M., Olver, R.E., 1996. Nebulised antipseudomonal antibiotic therapy in cystic fibrosis: a meta-analysis of benefits and risks. Thorax 51, 364–368.

Newman, S.P., Clarke, S.W., 1983. Therapeutic aerosols. 1. Physical and practical considerations. Thorax 38, 881–886.

O'Callaghan, C., Barry, P.W., 1997. The science of nebulised drug delivery. Thorax 52 (Suppl. 2), S31–S44.

Ramsey, B.W., Pepe, M.S., Quan, J.M., Kelly, L.O., Monygomery, A.B., Williams-Warren, J., Vasiljev-K, M., Borowitz, D., Bowman, C.M., Marshall, S., Smith, A.L., 1999. Intermittent administration of inhaled tobramycin in patients with cystic fibrosis. N. Engl. J. Med. 340, 23–30.

Touw, D.J., Brimicombe, R.W., Hodson, M.E., Heijerman, H.G.M., Bakker, W., 1995. Inhalation of antibiotics in cystic fibrosis. Eur. Respir. J. 8, 1594–1604.

Touw, D.J., Jacobs, F.A.H., Brimicombe, R.W., Heijerman, H.G.M., Bakker, W., Breimer, D.D., 1997. Pharmacokinetics of aerosolized tobramycin in adult patients with cystic fibrosis. Antimicrob. Agents Chemother. 41, 184–187.

Webb, A.K., Dodd, M.E., 1997. Nebulized antibiotics for adults with cystic fibrosis. Thorax 52 (Suppl. 2), S69–S71.

Weber, A., Morlin, G., Cohen, M., Williams-Warren, J., Ramsey, B., Smith, A., 1997. Effect of nebulizer type and antibiotic concentration on device performance. Pediatr. Pulmonol. 23, 249–261.