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International Journal of Pharmaceutics 189 (1999) 197–204

international journal of pharmaceutics

www.elsevier.com/locate/ijpharm

Influence of the technological parameters of ultrasonic nebulisation on the nebulisation quality of α1 protease inhibitor (α1PI)

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Received 7 April 1999; received in revised form 7 June 1999; accepted 19 July 1999

Abstract

The principle of an ultrasonic nebuliser is based on the vibrations of a piezo-electric crystal driven by an alternating electrical field. These periodical vibrations are characterised by their frequency, their amplitude and their intensity which corresponds to the energy transmitted per surface unit. When the vibration intensity is sufficient, cavitation appears and generates droplets. Ventilation enables an airflow to cross the nebuliser and to expulse the aerosol droplets. For a given nebuliser, the vibration frequency of the piezo-electric crystal is fixed and is often in the range of 1–2.5 MHz. In most cases, an adjustment in vibration intensity is possible by modifying vibration amplitude. The ventilation level is adjustable. The influence of these two parameters on the efficiency of ultrasonic nebulisation is studied. The study was carried out with a protein solution that had to be administered into the lungs. The solution used presented a viscosity of 1.25 mPa and a surface tension of 53 mN/m. The integrity of the protein was checked which was submitted to different vibration conditions. Nebulisation efficiency was evaluated by determining droplet size, the percentage of drug nebulised and nebulisation time. An increase in vibration intensity does not modify the size of droplets emitted, but decreases nebulisation time and raises the quantity of protein nebulised, thus improving performance. On the other hand, an increase in ventilation increases the size of droplets emitted, decreases nebulisation time and the quantity of protein nebulised because more drug is lost on the walls of the nebuliser. High intensity associated with low ventilation favours drug delivery deep into the lungs.

Keywords: α1 Protease inhibitor; Ultrasonic nebulisation; Intensity; Ventilation; Nebulisation quality

1. Introduction

Drugs administered to the lower part of the lungs as dispersions of solid or liquid particles in a gas require one of the following devices:

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0378-5173/99/$ - see front matter
PII: S0378-5173(99)00248-3

nebuliser
metered dose pressurised inhaler
dry powder inhaler

Nebulisers offer advantages: higher doses can be administered deep into the lungs, any incompatibilities between drugs and propellants and the problem of coordination between inhalation and activation can be avoided.

Two types of nebulisers are commonly used, each based on a different principle: jet nebulisers and ultrasonic nebulisers.

As part of a study of α1 protease inhibitor (α1PI) for pulmonary administration by jet nebulisation, the influence of formulation and technological parameters was studied, that is to say the nebuliser and dynamic conditions (airflow and pressure) on nebulisation quality. The results show that it seems very important not only to define and justify the formulation but also to associate with it the proper nebuliser(s) and conditions of administration (Flament et al., 1997).

Ultrasonic nebulisation can also be considered to administer α1PI to the lungs. This is usually applied via intravenous route for the treatment of pulmonary emphysema and is under consideration for cystic fibrosis.

The principle of an ultrasonic nebuliser is based on the vibrations of a piezo electric crystal (transducer) driven by an alternating electrical field. Ultrasounds are sound waves with a frequency higher than 20 000 Hz (Mercer, 1973; Clarke, 1988).

A sound wave is a periodical disturbance in a material medium, in which certain molecules are momentarily displaced from their equilibrium positions and experience a restoring force due to the elasticity of the medium. This restoring force is responsible for the propagation of the disturbance wave in the form of an oscillation of the molecules around their mean position, and its magnitude influences the velocity with which the wave is propagated. The propagation of sound waves through the medium involves alternating positive and negative deviation from the mean values of density, pressure, temperature, particle velocity and particle acceleration. If the pressure amplitude is sufficiently high, cavitation is very significant. This is the formation and collapse of small bubbles in the liquid. The negative pressure portion of the sound wave causes some of the vapour of the liquid to be emitted from the solution in the form of minute bubbles. These bubbles then act as weak spots where the liquid is torn apart to form larger cavities. Then when the pressure becomes positive, in the other half of the sound wave cycle, the cavities collapse with violent hammering which generates high local instantaneous pressures and temperatures. During the implosion of the bubbles, the instantaneous particle velocities reach supersonic speed and a tiny shock wave is produced and results in a water geyser at the surface. Periodic hydraulic shocks set the surface of the liquid into vigorous oscillatory motion, with the formation of standing capillary waves of finite amplitude on its surface and the spontaneous excitation of the standing capillary wave. If vibration intensity is sufficiently high, the amplitude of the capillary waves will also grow and their shape will increasingly deviate from that of a sine wave due to non-linearities. Finally, at high amplitudes, droplets will be propelled from the crests of the wave due to their instability and this leads to atomization of the liquid (Stahlhofen et al., 1983; Kane and Sternheim, 1986; Boguslavskii, 1995).

During ultrasonic nebulisation, waves formed on the surface of the solution have a wavelength λ (Mercer, 1973):

$$\lambda = (8\pi\ \gamma/\rho f^2)^{1/2}$$

where f is the frequency of the ultrasonic vibrations and γ and ρ are the surface tension and density of the liquid. The diameter of the droplets formed from the waves is:

$D = \alpha\lambda$ where α is a proportionality coefficient.

For a given nebuliser, the vibration frequency of the transducer is fixed and is often in the range 1–2.5 MHz.

For a given vibration frequency, the intensity of the wave is proportional to the square of wave amplitude (a) and its frequency (f):

$$I = kw^2a^2 \quad \text{with} \quad w = 2\pi f.$$

In most cases, an adjustment in vibration intensity is possible by modifying the vibration amplitude of the transducer.

Although air is not involved in the initial formation of droplets by ultrasonic vibrations, a flow of air is used to expulse the aerosol droplets. Ventilation, generally adjustable, sends an airflow through the nebuliser and carries out the aerosol produced.

The vibrations may be transmitted through a coupling liquid — commonly water — to a nebuliser cup containing the solution to be aerosolised (Mercer, 1973; Guichard, 1979; Lourenco and Cotromanes, 1992; Clarke, 1988; O'Doherty and Miller, 1993).

The cavitation phenomenon necessarily associated to droplet formation can lead to chemical modifications in ultrasonic nebulisation (Lepeschkin, 1949; Lourenco and Cotromanes, 1992).

The reactions initiated by ultrasonic waves may be macromolecule breakage or oxydoreduction reactions, that eventually lead to inactivation of the drug. Some authors relate these degradation reactions to an increase in temperature. (Marigulis, 1964).

The energy of acoustic oscillations can produce an excitation and a dissociation of water molecules, as free radicals ($^{\bullet}OH$) and ($^{\bullet}H$) are formed as well as electrons that can react with drugs (Marigulis, 1964; Mallol, 1993) causing especially oxydation and reduction. The water molecules can also react with dissolved oxygen and form hydrogen peroxide which is responsible for oxydation or hydrolysis (e.g. polysaccharides) (Lourenco and Cotromanes, 1992).

Mechanical hydrodynamic effects can lead to breakages, in particular for macromolecules. Friction forces between some macromolecule units and the solvent molecules may appear because of the phase displacement of the vibration motion of each of them (El'Piner, 1964).

These forces are so high that they greatly exceed the strength of the chemical bond c–c, c=c, c=0. The stress arising in microregions causing relatively slight deformations is not distributed uniformly and may be concentrated at dispersed points, thus leading to breakage of separate covalent bonds of the macromolecules.

It seemed of interest, as a corollary to the jet nebulisation study, to consider the influence of the technological parameters of ultrasonic nebulisation on nebulisation quality.

2. Materials and methods

2.1. Materials

Five ml of a 2% aqueous solution of α1PI prepared from a concentrate of human origin (CRTS, Lille, France).

The protein has a molecular weight of about 54 000 Da and is denaturated in aqueous solution when the temperature is higher than 40°C (Burnouf et al., 1987; Vercaigne, 1987). It is stable till a temperature of 40°C. The solution presents a viscosity of 1.25 mPa measured with a capillary viscosimeter following the method that is described in the European Pharmacopoeia. The surface tension of the solution is 53 mN/m and is measured with a Lauda TD1 tensiometer as soon as the solution is obtained.

Ultrasonic nebuliser SAM LS (Système Assistance Medicale, Le Ledat, France), the vibration frequency of which is 2.4 MHz with variable vibration intensity and ventilation levels. The SAM LS is equipped with a device called 'Control Dose'. This is a hemispheric convex lid, placed above and partially covering the solution. This device prevents projections of solution on to the nebuliser walls.

Environmental temperature and relative humidity are maintained constant, that is to say at 20°C and 40–45%.

2.2. Method

The technological parameters studied are:

Vibration intensity: position 2–6.
Ventilation level that can be 25 ($V_{1/2}$) or 50 l/min (V_1).

2.3. Evaluation of nebulisation quality

Firstly, nebulisation quality is evaluated through the electrophoretic behaviour and the conservation of protein activity. Evaluation is performed on the dead volume of the solution submitted to ultrasounds, this solution being submitted to ultrasounds during the entire nebuli-

sation. It was previously checked that the activity of protein delivered in nebulised droplets was maintained.

The aim of the electrophoretic study is 2-fold: on the one hand to ensure that nebulisation does not modify the α1PI molecular weight, and on the other hand to check there is no separation during nebulisation. The protein solution contains α1PI associated with other proteins.

During nebulisation, separation of the constituents can be more particularly observed for those of lower molecular weight because of the preferential nebulisation of one of these.

α1PI acts by inhibiting elastase. This antielastase activity must then be maintained after nebulisation. Secondly, if the results of the two previous studies are satisfactory, evaluation can be made of droplet size and concentration, the quantity of α1PI nebulised and nebulisation time. Each result is the mean of three replicate measurements.

2.3.1. Polyacrylamide gel electropheresis

An 8–25% concentration gradient gel is used. One μl of the following solutions containing 1 μg of protein are studied:

The α1PI solution before nebulisation.
The α1PI solution remaining in the nebuliser after nebulisation at intensities 2, 4, 5 and 6.
THE REFERENCE IS A BLEND OF STANDARD MOLECULES WITH HIGH MOLECULAR WEIGHTS.

Thyroglobulin (MM = 669 kDa), Ferritin (MM = 440 kDa), Catalase (MM = 232 kDa), Lactate dehydrogenase (MM = 140 kDa), human serum albumin (MM = 67 kDa).

Electrophoretic migration lasts 40 min at a voltage of 400. Calculating the relative migration with regard to the solvent front (R_f) of the standard molecules makes it possible to determine the molecular weights of the components in the solution tested.

2.3.2. Dosage of antielastase activity

This consists in bringing α1PI, a titrated quantity of elastase and the elastase substrate together, the last of which is a synthetic substrate bound to paranitroanilide that absorbs light at 410 nm when it is released. When there is no α1PI, the elastase hydrolyses the substrate and then releases paranitroanilide that absorbs light at 410 nm. In the presence of α1PI reacting with part of the elastase, the quantity of paranitroanilide released and consequently absorbency at 410 nm decreases. Measurement of absorbency variation makes calculation of antielastase activity possible. The decrease in activity could be related to an increase in temperature, which is why the temperature of the dead volume is taken into consideration. It is measured with a thermometer (precision ± 0.1°C) put into the solution immediately at the end of the nebulisation.

2.3.3. Size and concentration of droplets

Aerosol size distribution emitted from α1PI solution is determined with a laser size analyser: Mastersizer (Malvern, Orsay, Paris). The solution is directly nebulised in the laser beam. After repeated testing, the measurement variation is 2.4%. The results are expressed as the percentage of droplets below 5.79 μm and the median diameter. The concentration of droplets in the air is evaluated by the obscuration percentage of the laser beam, the median size being similar for each ventilation.

2.3.4. Quantity of α1PI nebulised

This is obtained by dosing the amount remaining in the nebuliser by immunonephelometry with a 'Behring Nephelometric Analyser' whose precision is within 3%.

2.3.5. Nebulisation time

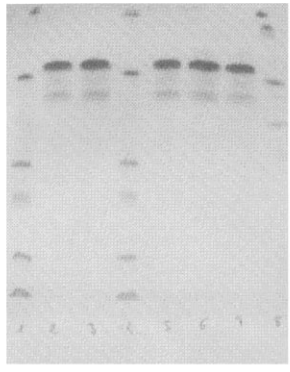
This parameter is important for patient compliance and must be taken into consideration (Aiache, 1973).

3. Results and discussion

The electrophoretic behaviour of the α1PI solution is similar before and after ultrasonic nebulisation (see Fig. 1).

For each deposit of α1PI solution, the following can be observed:

The α1PI band with a molecular weight of 54 kDa
A compound (albumin) with a molecular weight of 67–69 kDa.
A compound (haptoglobin) with a molecular weight of 77–82 kDa.
A compound with a molecular weight of 110–125 kDa.



Bands 1, 4 and 8 : references — Band 2 : before nebulisation
Band 3 : intensity 6 — Band 5 : intensity 4
Band 6 : intensity 5 — Band 7 : intensity 2

Fig. 1. Electrophoretic behaviour of the α1 protease inhibitor (α1PI) solution before and after ultrasonic nebulisation.

Table 1
Percentage of the different constituents of the α1 protease inhibitor (α1PI) solution before and after ultrasonic nebulisation at intensity 6

Compound	Before nebulisation (%)	After ultrasonic nebulisation (Intensity 6) (%)
α1PI	73.1	69.4
MM = 77–82 kDa (haptoglobin)	18.1	19.8
MM = 67–69 kDa (albumin)	5.6	6.3
MM = 110–125 kDa	3.2	4.5

The results of densitometric reading for the different deposits are very close. The results obtained for intensity 6 are compared to those of the solution before nebulisation (Table 1).

Table 2 presents the antielastase activity of the solution remaining in the nebuliser, the temperature at the end of nebulisation and the nebulisation time for different nebulisation intensities. The ventilation level used is 25 l/min.

The percentages of antielastase activity after nebulisation vary between 100.3 and 105.9%. The antielastase activity of α1PI is not modified during ultrasonic nebulisation, even when nebulisation time is extended. On the other hand, nebulisation time increases when nebulisation intensity decreases. The increase in temperature, related to nebulisation time, is moderate because of the specific design of the nebuliser used, in particular the presence of a coupling liquid. The coupling liquid absorbs the energy produced by ultrasonic vibrations and avoids the heating of the solution which often appears with ultrasonic nebuliser (Taylor and Hoare, 1993; Cipolla et al., 1994; Ip et al., 1995). The rise in temperature may be responsible for degradation of the drugs. For example, after ultrasonic nebulisation, Ip et al. (1995), reported a denaturation of a protein (recombinant methionyl interferon consensus) that could be avoided by preventing the heating of the solution. In the same way, Cipolla et al. (1994) noted an aggregation of rh DNase because of the elevation of temperature. In this case, the solution is not in contact with the piezo-electric crystal because of the presence of the coupling liquid that is why the present data differ from that previously reported.

Table 3 indicates the percentage of droplets below 5.79 μm, the percentage of α1PI nebulised and the nebulisation time when vibration intensity and ventilation vary. The results obtained with intensities of more than 4 are reported because at intensity 2, the mist emitted is limited making analysis difficult.

For a given intensity, an increase in ventilation increases obscuration percentage indicating a higher droplet concentration in the air and so a higher output rate, which is proved by the decrease in nebulisation time. Droplet size increases because of the aggregation of droplets due to

Table 2
Comparison of antielastase activity, nebulisation time and the temperature of the α1 protease inhibitor (α1PI) solution before and after ultrasonic nebulisation at different intensities, with a ventilation level of 25 l/min. Each result is the mean (+/-SE) of three experiments

Solution	Antielastase activity* (%)	Nebulisation time (min)	Temperature of the solution at the end of nebulisation (°C)
Before nebulisation	100 ± 0.43	–	20 ± 0.1
After nebulisation (Intensity 2)	100.3 ± 1.92	45 ± 1.5	28 ± 0.1
After nebulisation (Intensity 4)	101.4 ± 1.09	30 ± 0.1	26 ± 0.1
After nebulisation (Intensity 5)	105.5 ± 0.61	28 ± 1	25 ± 0.1
After nebulisation (Intensity 6)	102.8 ± 0.64	20 ± 0.10	25 ± 0.1

* Precision of the method: 8%.

higher concentration associated with higher turbulence in the airstream. The quantity of α1PI nebulised decreases with the ventilation level because more drug is lost to the wall of the nebuliser. Those results are in agreement with those of Taylor and Hoare (1993). They observed that operating the nebuliser at maximum setting decreased the time required to nebulise pentamidine isethionate solution but also decreased the efficiency of delivery.

The results show that an increase in intensity causes the formation of more mist in the nebulisation chamber leading to an increase in obscuration and in the quantity of α1PI nebulised as well as decrease in nebulisation time. For a given ventilation level, an increase in intensity does not modify the size of the droplets emitted because even if droplet concentration increases, there is no modification as regards turbulence in the airstream and consequently hazards of impact between particles. These observations are confirmed by the equation providing the diameter of the particles formed, which shows it is proportional to the wavelength of the waves formed and so to frequency but not to vibration amplitude.

For a given ventilation level, the increase in intensity does not modify droplet size but increases the quantity of drug nebulised and so the

Table 3
Influence of nebulisation intensity and ventilation level on the% of droplets <5.79 μm, quantity of α1 protease inhibitor (α1PI) nebulised, nebulisation time and % obscuration[a]

Intensity and ventilation level	Droplets < 5.79 μm (%)	Median diameter (μm)	α1PI nebulised (%)	Nebulisation time (min)	Obscuration (%)
Intensity 4					
V1/2	86.89 ± 2.51	1.64 ± 0.065	55.22 ± 2.08	30 ± 0.1	0.13 ± 0.001
V1	35.02 ± 2.5	9.13 ± 0.70	43.00 ± 1.6	25 ± 0.8	2.59 ± 0.25
Intensity 5					
V1/2	82.47 ± 1.72	1.66 ± 0.12	62.99 ± 1.10	28 ± 1	0.21 ± 0.025
V1	38.85 ± 2.88	9.18 ± 0.74	46.93 ± 1.23	23 ± 0.25	6.51 ± 0.6
Intensity 6					
V1/2	84.70 ± 1.53	1.66 ± 0.05	72.85 ± 2.71	20 ± 0.10	1.90 ± 0.10
V1	36.68 ± 0.89	9.97 ± 0.31	58.70 ± 2.64	17 ± 1	13.34 ± 0.5

[a] Each result is the mean (± S.E.) of three experiments.

quantity of drug likely to reach the lungs. Knowledge only of the size of emitted droplets is not sufficient to forecast nebulisation efficiency. It must be associated with the quantity of drug nebulised and nebulisation time.

The use of ultrasonic nebuliser SAM LS at nebulisation intensity 6 and a ventilation level of 25 l/min makes it possible to nebulise the α1PI solution satisfactorily as regards the size of droplets emitted, the quantity of α1PI nebulised and nebulisation time. These conditions make it possible to obtain results close to those of some jet nebulisers used with optimum conditions of airflow and pressure, as for example the Rotaneb Na 10, the Euroneb RN 300 (Europe Medical UK, Fleet, UK), the Optineb (Medic Air, Pagham, Sussex, UK) and the Pari Lc (Pari, Starnberg, Germany) (Flament et al., 1997).

A ventilation level of 25 l/min is to be preferred, on the one hand for the emission of the smaller size droplets required to administer the drug deep into the lungs and on the other hand for better compatibility with patient administration.

For a given ultrasonic nebuliser, the vibration frequency of the transducer is fixed. Only the vibration intensity and the ventilation level have to be defined for the nebulisation of a given solution with an ultrasonic nebuliser. On the contrary, with jet nebulisation a nebuliser with given geometric characteristics has to be chosen: liquid and air tube orifice diameters, size and shape of the reservoir, shape and position of the impaction system, and these have to be associated with airflow and pressure conditions to be defined. On the other hand, ultrasonic nebulisation gives a nebulisation quality comparable to that of jet nebulisation in defined conditions.

4. Conclusion

The compatibility of the ultrasonic vibrations of the SAM LS nebuliser with α1PI has been shown. When the α1PI solution is submitted to these ultrasonic vibrations, there is no modification of molecular weight and the antielastase activity of the protein is maintained.

This nebuliser also offers the advantage of using a coupling liquid that transmits ultrasonic vibrations to the solution and limits any increase in temperature. This is particularly interesting for thermosensitive substances which could be used with this type of nebuliser.

To obtain a constant quantity of drug for the patient at each administration, it is necessary to standardize operating conditions: vibration intensity and ventilation level have to be defined. High vibration intensity associated with a low ventilation level is preferable for the delivery of drugs deep into the lungs.

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