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Ultrasonic surfactant nebulization with different exciting frequencies

M.H. Wagner [a,*], S. Wiethoff [b], W. Friedrich [c], I. Mollenhauer [b], M. Obladen [a], U. Boenick [b]

[a] *Department of Neonatology, Charité, Campus Virchow-Klinikum, Humboldt-University, Augustenburger Platz 1, 13 353 Berlin, Germany*
[b] *Institute for Biomedical Engineering, Technical University, Berlin, Germany*
[c] *Department of Neonatology, Charité, Campus Mitte, Humboldt-University, Berlin, Germany*

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Abstract

Intratracheal bolus instillation of natural lung surfactant is the treatment of choice in neonatal respiratory distress syndrome and an increasing part in adults' therapy. For reasons of hemodynamics, surfactant distribution and efficiency the application mode should be improved. Nebulization seems to have some advantages but its technical realization is difficult. The aim of the present study was to investigate if ultrasonic nebulization with exciting frequencies higher than 2.8 MHz can improve the efficiency of surfactant nebulization without changing the surface-active properties of the material. Exciting frequencies of 1.7, 3.3 and 4.0 MHz were used to produce a surfactant aerosol. The phospholipid content in the liquefied aerosol and particle size distinctly dropped with higher frequencies. The surface activity was not altered in the produced aerosol and neither in the surfactant remaining in the nebulizer. Although possible, ultrasonic nebulization of surfactant suspensions is ineffective because of a striking decrease in phospholipid content.

Keywords: Natural surfactant; Nebulization; Nebulization frequency; Phospholipids; Surface activity; Droplet size

* Corresponding author. Tel.: +49-30-45066122; fax: +49-30-45066922.
E-mail address: mathias.wagner@charite.de (M.H. Wagner)

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1. Introduction

Surfactant, a phospholipid–protein complex secreted into the fetal lung, facilitates lung expansion and allows functional residual volume after birth. In premature infants with lack of this substance, natural surfactant preparations are substituted to avoid or treat respiratory distress syndrome [1–3]. Lung instillation of the — mostly fluid — substance is done commonly by a bolus injection into the intratracheal tube of the ventilated patient. The effect of a better lung inflation occurs rapidly and improves oxygenation within minutes. However, there are some reasons why researchers are looking for other instillation methods and especially focussing on aerosolization of surfactant: (1) some undesired side effects of surfactant bolus instillation could be observed, such as drop in blood pressure, changes of cerebral blood flow velocity and a reduction in EEG activity [4–10]. Speculations were raised about a possible association with cerebral hemorrhages, but could not be proved [7,11]. (2) Distribution of nebulized, inhaled surfactant is superior to bolus-instilled surfactant, as it results in lower doses and better effects [12–14]. No side effects have been observed [8,13–15]. (3) Already established in neonatology, this therapy could be successfully applied in adult respiratory distress syndrome (ARDS), if the more physiologic aerosol application leads to a better acceptance in adults anesthesia. Until now, concerns still exist regarding the excessive fluid load of the lung and the high costs in ARDS therapy [14,16].

However, it is difficult to nebulize surfactant effectively, economically and safely without destroying the surface-active properties of the substance. Nebulization is characterized by a massive loss of material within the nebulizer system and the upper airways, until now no more than 10% of the nebulized material could be recovered in the lung [8,12–15,17,18]. Studies on nebulization are hard to compare because of different techniques (jet, ultrasonic) and different surfactants (natural, artificial). Few, but conflicting reports exist about the surface activity and the effect of surfactant after ultrasonic nebulization [14,17–22]. Nevertheless, particularly in neonatology, small airways and breathing volumes make ultrasonic nebulizers appear advantageous compared to jet nebulizers. Working as a demand system, they do not produce additional airflow and pressure in the ventilation system, work with low noise and high humidity [23–25]. Droplet size and aerosol densities are responsible for the deposition rate and site of an aerosolized substance. According to Mathieu's differential equation, the droplet size of an aerosol produced by ultrasound, depends on the capillary wavelength of the nebulized substance and subsequently from the ultrasonic (or exciting) frequency [26–28].

The aim of this study was:

1. to investigate the efficiency of ultrasonic surfactant nebulization by applying frequencies of 3.33 and 4 MHz in comparison to 1.8 MHz, which is commercially used in medical nebulization; and
2. to examine if this procedure influences phospholipid content or surface active properties of the aerosol.

2. Material and methods

Commercially available ultrasonic nebulizers use almost exclusively exciting frequencies below 3 MHz. In order to increase the density of the aerosol, by accepting lower particle sizes, two piezoelectric ceramic discs (PZ 27, Ferroperm, Kvistgård, Denmark) with diameters of 22.5 mm and heights of 2.117 and 1.754 mm were excited by their third parallel resonance frequency, so that nebulization occurred at frequencies of 3.33 and 4 MHz. Additionally, the ultrasonic field was focussed by an acoustic lens casted for this purpose from an epoxide resin with a curvature radius of 25 mm. For comparison an ultrasonic nebulizer MEDAP U 804 (Medizinische Apparate GmbH, Usingen, Germany) with a frequency of 1.7 MHz was used.

The porcine surfactant Curosurf® (Serono, Unterschleißheim, Germany) consists of 99% polar lipids (75 mol% posphatidylcholin, 4 mol% phophatidylglycerol) and 1% surfactant proteins

B and C (ratio approx. 1:3). Neutral lipids and hydrophilic proteins A and D are absent. The usual concentration of 80 mg/ml was diluted with normal saline to approximately 20 mg/ml by sonication on ice (5 min, 35 kHz; Sonorex TK52, Bandelin Elektronic, Berlin, Germany) [25]. No changes of the surface active properties of the substance occurred, which was proved by bubble surfactometer (data not shown).

Fig. 1 shows the experimental design for nebulization with frequencies of 3.33 and 4 MHz. The Mass Median Aerodynamic Diameter (MMAD) of the aerosols was determined by a cascade impactor type 'Berner' (Hauke GmbH, Gmunden, Austria) for all frequencies. In a second experiment the cascade impactor was replaced by a thin glass tube on ice for condensation. The then collected liquefied aerosol was examined for its phospholipid content, as well as for its surface active properties. The results were compared with the values of the original surfactant suspension before nebulization and with the residual suspension in the nebulizer storage chamber after nebulization. Phospholipid content was determined according to Morrison [29]. Surface activity was measured by a pulsating bubble surfactometer (Electronetics, Amberst, NY, USA). First measurement was made statically at minimum bubble size after an adsorption time of 10 s (γ_{min}), then bubble cycling was started at 20 cycles/min and surface tension was determined at 20 s and 5 min for minimum as well as maximum bubble size (γ_{max}) [30–32].

We performed eight nebulizations for each frequency. The nebulizer reservoir was refilled from the same surfactant suspension pool when the experiment took place the same day. All values are given as means ± S.D. PL content was determined in eight samples before and after each nebulization. In the reservoir remnant we measured the PL content in six samples for 1.7 and 3.3 MHz and in seven samples for 4 MHz, the other specimen were used to investigate the influence of longer nebulization times. PL content was determined twice in each sample and the mean was taken as the result for this nebulization performance. Surface tension, γ, was determined in all samples before nebulization ($n = 8$). In the liquefied aerosol, surface active properties were investigated in eight samples at 1.7 MHz and in five samples at 3.3 and 4 MHz. Some of the post-nebulization samples, with higher frequencies, had such low PL concentrations that a determination of surface tension was impossible. For the same reason the samples of each nebulization experiment were diluted with normal saline to an equal concentration in order to make surface tension values comparable. Therefore, the samples for the 1.7 MHz nebulization were measured at 2 mg PL/ml and the samples of the 3.3

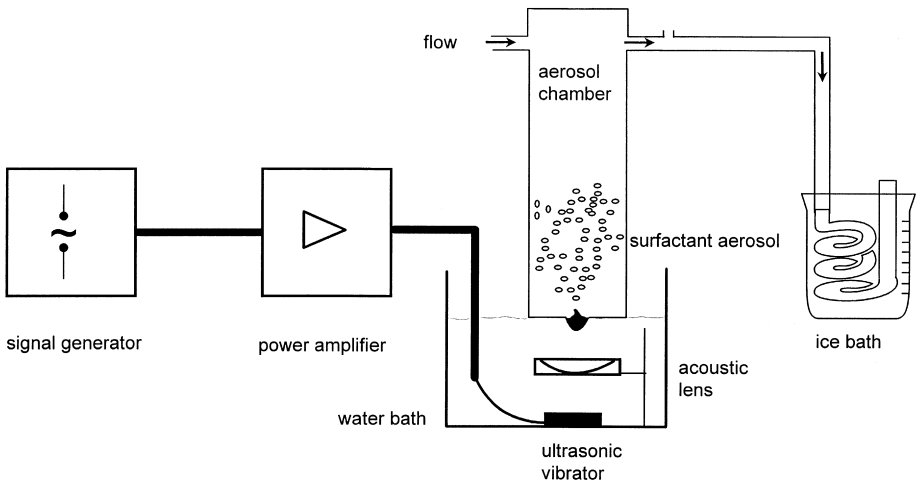

Fig. 1. Schematic experimental design for ultrasonic nebulization with 3.3 and 4 MHz and determination of droplet size.

and 4 MHz nebulization at 0.5 mg PL/ml. The same was done with the samples of the reservoir remnant with $n = 6$ for 1.7 and 3.3 and $n = 7$ for 4 MHz, the other specimens were used for the experiments with longer nebulization times.

3. Results

3.1. Phospholipid content

After nebulization the PL content in the liquefied aerosol decreased markedly in comparison to the PL content of the surfactant suspension before nebulization (Fig. 2). During 1.7 MHz nebulization, the concentration of PL decreased from 20.38 ± 3.63 mg/ml before to 2.74 ± 2.23 mg/ml (10.4%, $P = 0.0117$) after nebulization. Using the higher frequency of 3.3 MHz the PL content of the samples dropped from 19.74 ± 2.62 to 0.84 ± 0.64 mg/ml (4.2%, $P = 0.0117$). Even worse was the result with 4 MHz: 19.44 ± 3.41 mg/ml PL before and 0.49 ± 0.58 mg/ml PL after nebulization (2.4%, $P = 0.0117$). In contrast, the PL content of the remaining suspension in the nebulizer reservoir increased to 22.24 ± 3.14 with 1.7 MHz (n.s.), to 28.52 ± 5.21 with 3.3 MHz ($P = 0.0464$), and to 27.23 ± 3.36 with 4 MHz nebulization ($P = 0.018$).

4. Surface tension

Table 1 and Fig. 3 show the surface active properties at the three different frequencies both before and after nebulization. The samples of liquefied aerosol showed less adsorption with an approximately doubled surface tension after 10 s of static measurement ($P = 0.027$ for 1.7 MHz and $P = 0.043$ for 3.3 and 4 MHz), whereas the samples of the remnant did not change adsorption behavior. After 20 s differences for γ_{min} as well as for γ_{max} were significant only for the samples of liquefied aerosol and highest frequency of 4 MHz ($P = 0.043$ for both), no changes for the remnant samples could be determined. After 5 min no significant differences for γ_{min}, and γ_{max}, could be found in samples before nebulization and liquefied aerosol, but the surface tension was significantly lower for the remnant

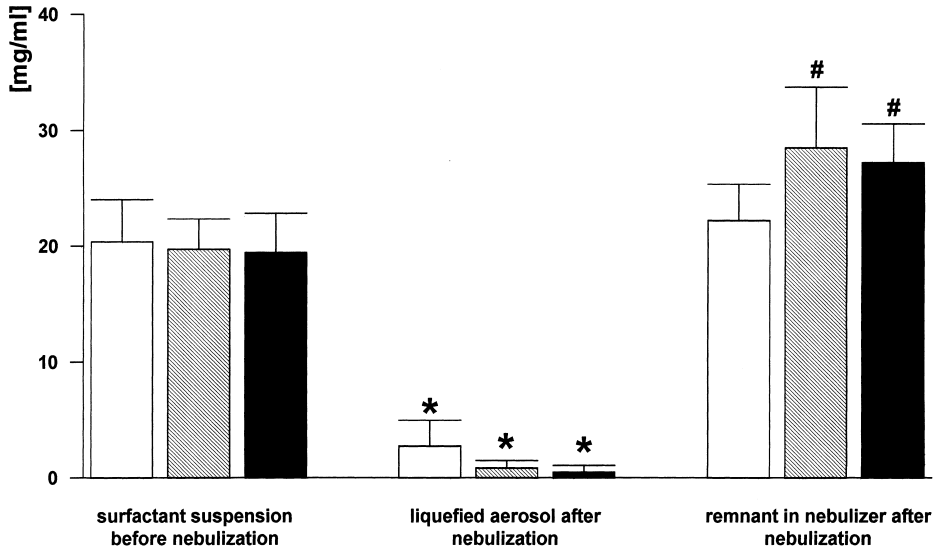

Fig. 2. PL content of the surfactant suspension before ($n = 8$), the liquefied aerosol after ($n = 8$) and the remnant in nebulizer reservoir (1.7/3 MHz $n = 6$; 4 MHz $n = 7$) after nebulization with 1.7 MHz (open columns), 3.3 MHz (shaded columns) and 4 MHz (black columns). P-levels < 0.05 are indicated with * for differences between the suspension before and after nebulization and with # for differences between the suspension before nebulization and the remnant in nebulizer.

Table 1
Surface tension measurements[a]

Nebulization frequencies	1.7 MHz		3.33 MHz		4.0 MHz	
Nebulization time	0 min original suspension ($n = 8$)	20 min liquefied aerosol ($n = 8$)	0 min original suspension ($n = 8$)	35 min liquefied aerosol ($n = 5$)	0 min original suspension ($n = 8$)	35 min liquefied aerosol ($n = 5$)
γ After 10 s (mN/m)	26.98 ± 1.83#	44.74 ± 17.39	26.25 ± 0.54#	50.54 ± 14.43	26.79 ± 5.36#	58.12 ± 9.69
γ_{min} After 20 s (mN/m)	14.33 ± 4.58	28.49 ± 16.91	16.80 ± 2.32	26.28 ± 12.82	18.41 ± 3.68#	37.86 ± 13.47
γ_{max} After 20 s (mN/m)	35.39 ± 3.52	51.06 ± 17.87	33.84 ± 0.39	56.72 ± 14.62	34.66 ± 1.72	63.7 ± 9.17
γ_{min} After 5 min (mN/m)	9.23 ± 4.90	14.8 ± 12.29	10.61 ± 1.98	16.96 ± 9.6	11.65 ± 5.87	21.36 ± 9.34
γ_{max} After 5 min (mN/m)	39.0 ± 6.52	51.79 ± 13.56	44.26 ± 1.09	53.36 ± 12.46	43.21 ± 3.17	57.9 ± 9.9

[a]Surface tension after adsorption, minimal and maximal surface tension after 20 s and 5 min in the original suspension, and in the liquefied aerosol after 20 min nebulization with 1.7 MHz, and 35 min with 3.3 and 4 MHz. Values are mean ± S.D. *P*-level 0.05 for differences between suspension before and after nebulization is marked with #.

samples after 3.3 ($P = 0.028$) and 4 MHz ($P = 0.042$).

Surface tension of the remnant in the nebulizer reservoir was determined after influence of ultrasonic waves with different frequencies from 0 to 120 min. No negative effect on surface tension could be found, not even with the highest frequency of 4 MHz and longest time of 120 min (data not shown).

5. Particle size

The particle size of the produced aerosols measured as Mass Median Aerodynamic Diameter distinctly decreased from 4.98 μm at 1.7 MHz nebulization to 0.47, μm respectively, 0.48 μm with the higher frequencies of 3.3 and 4 MHz.

6. Discussion

6.1. Methods

Although surfactant bolus instillation is an established method for the treatment of RDS in neonatology, the search for improved or even alternative application methods continues [5,8,12–15,18]. Nebulization seems to be promising and ultrasonic nebulization may have some advantages in comparison to jet nebulization — aerosol is produced without additional flow or pressure on a demand principle in the ventilation system, which is particularly important for premature infants [23,24].

The aerosol generation by ultrasound is not a thermic process, rather the aerosol is generated by capillary waves whose amplitude determines the intensity of nebulization and whose wavelength correlates with the droplet size [27]. We decided to use exciting frequencies higher than those of commercially available ultrasonic nebulizers in order to enhance the effectiveness of nebulization by increasing the aerosol density and at the same time admitting a small droplet size. The range of the droplet size consequently is on the lower edge of medical aerosols.

Furthermore, we investigated whether natural surfactant is altered in surface active properties or not. Since the study intended to test a commercially available surfactant, which is also in clinical use, economical considerations were of importance too. Therefore, the nebulization times are different for 1.7 MHz and the higher frequencies due to different collecting times for the samples. A pulsating bubble surfactometer was chosen to determine the surface active properties since this method enables measurements of very small sample volumes with sufficient precision [30–32].

6.2. Results

In this study, surface tension of a surfactant

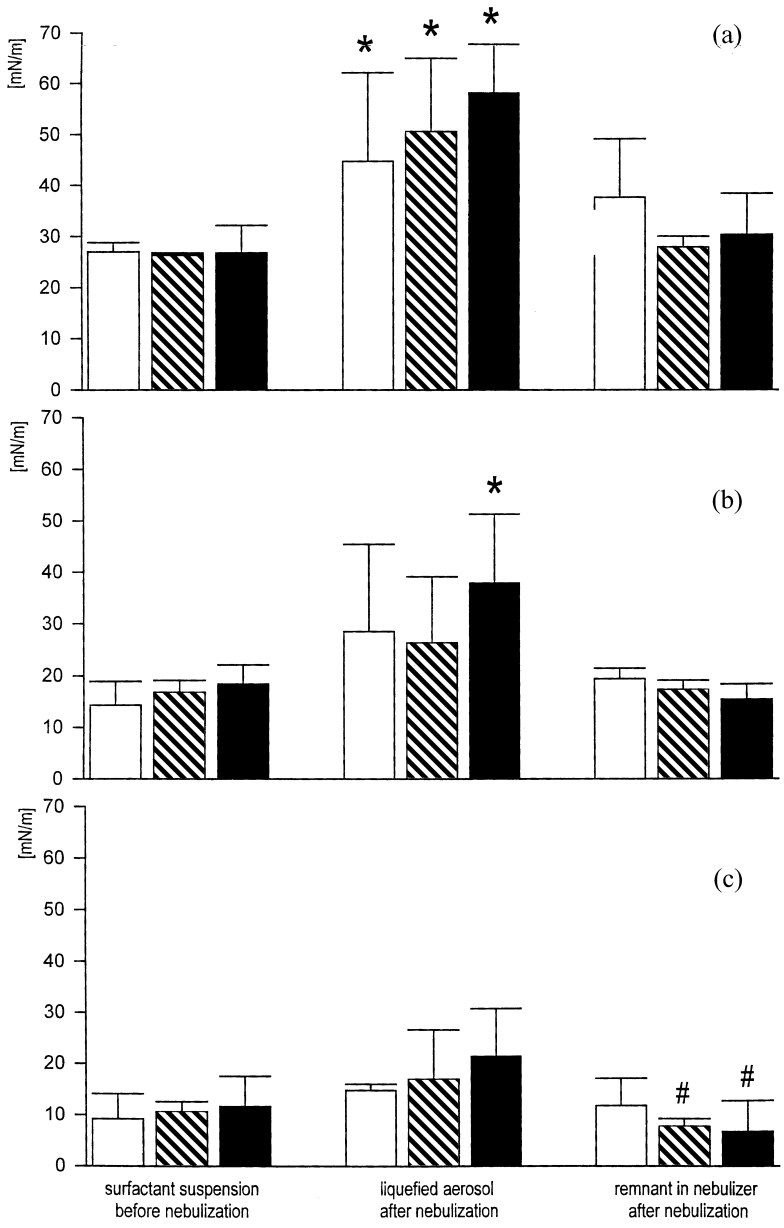

Fig. 3. Surface tension measured in bubble surfactometer after adsorption time (a), 20 s (b), and 5 min (c) for surfactant suspension before ($n = 8$), liquefied aerosol after (1.7 MHz $n = 8$, 3.3/4 MHz $n = 5$), and remnant in nebulizer reservoir (1.7/3 MHz $n = 6$, 4 MHz $n = 7$) after nebulization with 1.7 MHz (open columns), 3.3 MHz (shaded columns) and 4 MHz (black columns). *P*-levels < 0.05 are indicated with * for differences between the suspension before and after nebulization and with # for differences between the suspension before nebulization and the remnant in nebulizer.

suspension after nebulization was significantly higher only after adsorption time and for γ_{min} and γ_{max} after 20 s at 4 MHz. After achieving the steady state in the bubble surfactometer (γ_{min} after 5 min) a trend can be seen showing greater differences (although not significant), with higher nebulization frequencies. Looking at the reservoir remnant after nebulization this trend seems to be

vice versa — the higher the nebulization frequency, the lower the achieved surface tension in the reservoir remnant.

Different reports exist about the effect of ultrasonic waves on surfactant suspensions. Winsel et al. [20] found an increased surface tension due to lowered PL content in the aerosol as well as in the nebulizer remnant, which they explained by a chemical destruction of the phospholipids. Marks et al. [19] found unchanged surface active properties and adsorption characteristics of nebulized surfactant, but did not determine the PL content. Both authors used animal lung surfactant — from guinea pigs [20] and cattle [19]. Ikegami et al. [21] tested the clinical effect of surfactant in rats and found a very poor reaction on nebulized surfactant but an unchanged surface tension. Li et al. [17], in contrast, found a good response — surface tension was not influenced by exposure to ultrasound up to 30 min, but they tested only the remnant for surface activity in vitro, the effect of the aerosol was proved in vivo.

Efforts to design and test an artificial surfactant gave rise to the discussion about the effect of nebulization by ultrasound. It is proved that the effect of artificial surfactant greatly depends on preparation techniques and the morphological aspects of the produced material. Large unilamellar vesicles of artificial surfactant (mainly a phosphatidylcholin/phosphatidylglycerol mixture) are surface active but can be transformed to small unilamellar vesicles which are not surface active any more [22,33]. Fok et al. [18] recently tested natural and artificial surfactant in rabbits. They found no differences in clinical effects, but a slightly better deposition rate of artificial surfactant. In connection with our findings this could be explained by two different mechanisms: artificial surfactant is better nebulized by ultrasound, but the material is changed morphologically and therefore not active; in contrast, natural surfactant is more stable in its biophysical appearance, but so little material is nebulized that this cannot cause good clinical effects. These results confirm the findings of Winsel [20] of a very low PL content after ultrasonic nebulization and an unchanged surface activity — specially since the differences for γ_{min} after 5 min before and after nebulization are very small for all frequencies. Additionally, higher exciting frequencies produce, according to the physical principles, a smaller droplet size but the aerosolization could not be intensified to achieve a higher PL content. The increase of PL content in the remnant after nebulization indicates that mostly saline is aerosolized whereas the PL's stayed in the suspension. Although ultrasonic nebulization seems not to influence the surface active properties of natural surfactant, a raise of the exciting frequency does not help to solve the problem of effectiveness of surfactant nebulization. On the contrary, the lower frequency of 1.7 MHz appears to be advantageous because of the higher phospholipid content. We speculate that lower frequencies and higher capillary wavelengths, respectively, enable an easier detachment of the phospholipids out of the complex lipoprotein bonds of the surfactant solution. This phenomenon could explain the lower PL content of natural surfactant aerosols produced by higher frequencies and the better nebulization of artificial surfactants, since no lipoprotein bonds can stabilize the large unilamellar vesicles so that they are cleaved to small unilamellar vesicles which are not surface active.

Although probably not changing surface active properties, ultrasonic nebulization of surfactant with frequencies higher than 2.8 MHz does not have advantages compared to traditional nebulization. Further investigations should pay attention to the frequency of the used nebulizer and characterize the produced aerosol in vitro before blaming the loss of material within the nebulizer and ventilation system for impaired in vivo efficacy of surfactant.

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