

Intranasal Surfactant Aerosol Therapy for Otitis Media With Effusion

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Objective: To determine the effect of surfactant alone and with other medications delivered intranasally as a metered dose inhaler (MDI) aerosol on the resolution of experimentally induced otitis media with effusion (OME). **Background:** Eustachian tube dysfunction is a primary factor in the pathogenesis of OME. Intranasal surfactant via MDI has been shown in this laboratory to reduce passive opening pressure of the eustachian tube in normal gerbils and mice. **Study Design:** OME was developed in 35 gerbils by transtympanic injection of 10 μ g lipopolysaccharide from *Klebsiella pneumoniae*. Pretreatment otomicroscopy and tympanometry were performed to exclude pre-existing middle ear disease, and postinfection evaluations were performed on alternate days for a period of 30 days. Five animals received no treatment (control group); four were treated with propellant only (placebo); seven received surfactant alone; eight received surfactant and betamethasone; and six received surfactant with phenylephrine. All medications were sprayed intranasally as an aerosolized MDI and administered daily from postinfection day 2 onward. **Results:** OME resolved after 16.0 ± 0.44 days (mean \pm SD) in controls. There was no difference seen in the placebo or the surfactant with phenylephrine groups. Treatment with surfactant yielded resolution in 10.57 ± 0.37 days; this was reduced to 8.57 ± 0.37 days with surfactant plus betamethasone. These differences are statistically significant. There was no recurrence of OME in any group. **Conclusion:** This study demonstrates that using an aerosolized MDI surfactant with and without betamethasone decreases the duration of OME in this in vivo gerbil model. **Key Words:** Surfactant, otitis media with effu-

sion, gerbil, aerosolized metered dose inhaler, eustachian tube dysfunction.

Laryngoscope, 110:1857–1860, 2000

INTRODUCTION

Costs for medical and surgical interventions for treating otitis media with effusion (OME) approach \$3 billion annually in the United States.¹ Hearing loss, delays in speech and language development, and other complications may result from chronic otitis media.² Eustachian tube dysfunction is a primary factor in the pathogenesis of OME.³ Clinical and experimental studies show that eustachian tube dysfunction is characterized by obstruction with increased opening pressure.⁴ Failure to correct eustachian tube dysfunction results in persistent OME which, in turn, elevates the eustachian tube opening pressure independent of other pathological conditions of the tube.⁵

Lecithin (phosphatidylcholine with fatty acid residues of various chain lengths) is a phospholipid that normally exists in the eustachian tubes and middle ears of animals and humans that serves to lower the passive opening pressure of the eustachian tube.^{6–9} In an acute lung injury model, surfactant, the common form of lecithin, delivered via metered dose inhaler (MDI)¹⁰ is effective in reducing lung surface tension and restoring function.^{11–13} Surfactant functions as an ideal preparation, as it spreads rapidly over the eustachian tube mucosal surface to lower its lining layer surface tension, thereby allowing the tube to open more easily. Its effect is based on Laplace's law¹⁴ [pressure = 2(surface tension/radius)] which defines the forces needed to open the eustachian tube and prevent collapse. Statistically significant lowering of the passive opening pressure of the eustachian tube by aerosolized MDI surfactant has been demonstrated in our laboratory (Venkatayan N, unpublished data).

This experiment evaluates the effect of a synthetic surfactant delivered intranasally via a metered dose aerosol on the resolution of experimentally induced OME. It is the first study of its kind, demonstrating the applicability of an MDI surfactant aerosol to the intranasal delivery of therapeutic agents in any animal model.

Presented as a Poster at the 103rd Annual Meeting of the American Laryngological, Rhinological and Otological Society, Inc., Orlando, Florida, May 14–16, 2000.

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Editor's Note: This Manuscript was accepted for publication August 2, 2000.

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MATERIALS AND METHODS

Thirty-five gerbils (*Meriones unguiculatus*) were given experimental OME in the right ear and then divided into five study groups. These adult gerbils weighed between 51 and 60 g. Five animals succumbed to anesthetic complications and could not complete the experimental protocol. The remaining animals were divided into the following groups: control (no treatment) (n = 5 animals), placebo (propellant only) (n = 4 animals), surfactant alone (n = 7 animals), surfactant and betamethasone (n = 8 animals), and surfactant and phenylephrine (n = 6 animals).

The drugs were formulated and administered in the form of an aerosol in an MDI. Dosages were determined according to an average gerbil body weight of 55 g. Surfactant consists of dipalmitoylphosphatidylcholine and cholesteryl palmitate mixed in a ratio of 200:1 (w/w). Each MDI actuation delivers 5 mg of aerosolized surfactant. Surfactant plus betamethasone is formulated as 10 µg of betamethasone in 0.9 mg of surfactant. Surfactant plus phenylephrine is formulated as 90 µg of phenylephrine in 4.1 mg of surfactant. Each animal was anesthetized by an intraperitoneal injection of pentobarbital (50 mg/kg) and buprenorphine (0.03 mg/kg) for the initial examination and induction of OME (Venkatayan N, unpublished data).

Otomicroscopy was performed using a Zeiss microscope (model OpMi-1; Carl Zeiss, Inc., Thornwood, NY, U.S.A.). A modified Fulghum¹⁵ scale for grading otitis media was used in assessment of each ear (Table I). Tympanometry was completed using a Grason-Stadler (Milford, NH) GSI-1723 middle ear analyzer with a probe tone of 660 Hz, a sensitivity of 1.0, and a function setting of "Y" for compliance. All animals were evaluated visually and tympanometrically and found to have disease-free middle ears before beginning the experimental protocol.

Otitis media with effusion was induced into the right middle ear of each animal by intratympanic injection via a 28-gauge needle under microscopic guidance. Ten micrograms of lipopolysaccharide endotoxin derived from *Klebsiella pneumoniae* strain was injected into each right middle ear space. The left ear served as the unperturbed control. No constitutional symptoms were observed after intratympanic injection of lipopolysaccharide.

Two days after intratympanic injection each animal was anesthetized using an intraperitoneal administration of 75 mg/kg ketamine and 4 mg/kg xylazine ("gerbil cocktail"). Otomicroscopic examination and tympanometry were performed on both ears. OME was confirmed in the right ear of all gerbils with a grade of 3 or 4 on the modified Fulghum scale and a type B tympanogram. All left ears were normal.

Experimental therapies were administered once daily into the right nostril via MDI. Otomicroscopic examination and tympanometry were completed on alternate days for a period of 30 days under "gerbil cocktail" anesthesia. Nasal spray was discontinued when the animals were determined to be clinically normal

as determined by otomicroscopic examination. "Normal" otomicroscopy was defined as grade 0 on the modified Fulghum scale. Nevertheless, otomicroscopy and tympanometry were continued on alternate days for the remainder of the 30-day experimental period to observe any possible recurrence of OME. All experiments were conducted in accordance with the Institutional Animal Care and Use Committee and Institutional Review Board protocols.

To evaluate the effects of treatment on the duration of OME a one-way ANOVA was performed separately on otomicroscopy and tympanometry data using the number of days to normal as the dependent variable. The strength-of-association measure (ω^2) was calculated for the statistically significant main effect. Newman-Keuls posthoc multiple comparison tests were completed on all significant effects to determine statistically significant differences for all comparisons among the means. Significance was set as $P < .05$.

RESULTS

Otomicroscopy

Surfactant plus betamethasone treatment resulted in significantly shorter duration of effusion (mean \pm SD = 8.57 \pm 0.37 d) than all other treatments, control, and placebo ($P < .05$). Duration of effusion after treatment with surfactant alone (10.57 \pm 0.37 d) was significantly shorter than after treatment with surfactant plus phenylephrine, control, and placebo ($P < .05$). Mean OME duration was not statistically different for surfactant plus phenylephrine (15.67 \pm 0.52 d), control (16.0 \pm 0.44 d), or placebo (16.5 \pm 0.51 d).

These data are shown in Table II and Figure 1. Results of the ANOVA revealed statistically significant differences among the drug treatment group means ($F_{4,29} = 46.11$, $P < .05$). The ω^2 value for the effect of treatment as measured by otomicroscopy was 0.88. This indicates an extremely powerful effect of treatment on the duration of OME in that 88% of the experimental variance can be attributed to the treatment administered.

Nasal spray was discontinued on otomicroscopic resolution of OME. Otomicroscopy revealed no abnormal findings and no recurrence of OME for the remainder of the 30-day experimental period.

Tympanometry

Surfactant plus betamethasone treatment resulted in significantly shorter time to normal (type A) tympanogram (mean \pm SD = 12.29 \pm 1.60 d) than all other treatments, control, and placebo groups ($P < .05$). Tympanometric resolution after treatment with surfactant alone (15.71 \pm 1.19 d) was significantly shorter than after surfactant plus phenylephrine, control, and placebo ($P < .05$). Mean OME duration assessed by tympanometry was not statistically different for surfactant plus phenylephrine (28.33 \pm 0.52 d), control (28.0 \pm 0.44 d), or placebo (28.54 \pm 0.51 days). These data are shown in Table II and Figure 2. Results of the ANOVA revealed statistically significant differences among the drug treatment group means ($F_{4,29} = 42.83$, $P < .05$). The ω^2 value for drug treatment effect as measured by tympanometry was also 0.88.

Although tympanometry remained abnormal longer than otomicroscopic examination ($P < .05$), the courses of OME resolution as assessed by either technique were par-

TABLE I.

Otomicroscopy Grading Scale for Otitis Media With Effusion.

Observation	Assessment of OME Status	Grade
TM clear	Normal	0
TM hyperemic	Very mild	1
TM cloudy, ossicles faintly seen	Mild	2
TM cloudy and retracted	Moderate	3
TM bulging and exudates present	Severe	4

Modified from Fulghum.¹⁵
TM = tympanic membrane.

TABLE II.
Days to Resolution of Otitis Media With Effusion.
Mean (\bar{X}), Standard Error (SE) and number of animals (n) to days for resolution of OME in each study group as measured by Otomicroscopy and Tympanometry.

Study Group	Otomicroscopy			Tympanometry		
	\bar{X} days	SE days	n	\bar{X} days	SE days	n
Control	16.00	0.44	5	28.00	0.44	5
Placebo	16.50	0.51	4	28.54	0.51	4
Surfactant	10.57*	0.37	7	15.71*	1.19	7
Surfactant + betamethasone	8.57*	0.37	8	12.29*	1.60	8
Surfactant + phenylephrine	15.67	0.52	6	28.33	0.52	6

*Statistically significant: $P < .05$.

allel (Fig. 3). Once achieving normal configuration, tympanometry remained normal after cessation of treatment, during the remainder of the 30-day experimental period.

DISCUSSION

The eustachian tube is a compliant liquid-lined tube with the resulting surface tension directed toward collapsing of the lumen. Greater transluminal pressure is necessary to maintain patency when the surface tension inside the eustachian tube is higher, as defined by Laplace's law. OME is known to elevate the eustachian tube opening pressure independent of other pathological conditions of the tube.⁵ Surfactant acts to reduce surface tension, thereby decreasing the passive opening pressure of the eustachian tube and counteracting the OME effect.

This animal study clearly demonstrates that intranasal aerosolized MDI treatment of OME with surfactant alone or in combination with betamethasone results in a significantly shorter time to resolution of OME as assessed by both otomicroscopy and tympanometry. In prior experiments, we have shown that intranasal aerosolized MDI surfactant reduces the passive opening pressure of the eustachian tube and maintains its patency. Intranasal surfactant alone, therefore, must facilitate drainage of the effusion. In the case of surfactant administered in con-

junction with betamethasone, we hypothesize that the surfactant opens up the eustachian tube, allowing the betamethasone to spread along the eustachian tube quickly to arrest the tissue component of the inflammation in the middle ear. Treatment effect cannot be attributed to the use of the aerosolized MDI, as the placebo was delivered via an MDI and showed no difference in outcome compared with the control group.

The duration of OME was not influenced by treatment with surfactant in combination with phenylephrine relative to the control and placebo. The draining function of the surfactant appears to have been negated by the vasoconstrictive and decongestive properties of the phenylephrine. We presume that phenylephrine dries the mucosal lining of the eustachian tube and thus retards the spread of surfactant.

The placebo and the control groups reverted to normal despite no treatment as a result of their normal eustachian tube function and the self-limiting, localized nature of experimentally induced OME. Even when the treatments were discontinued, there was no recurrence of OME, indicating no need for prolonged or prophylactic therapy.

It is interesting that effects of treatment on the resolution of the OME were identical whether the animals were evaluated by otomicroscopy or tympanometry. However, the time course of the OME was shorter as evidenced

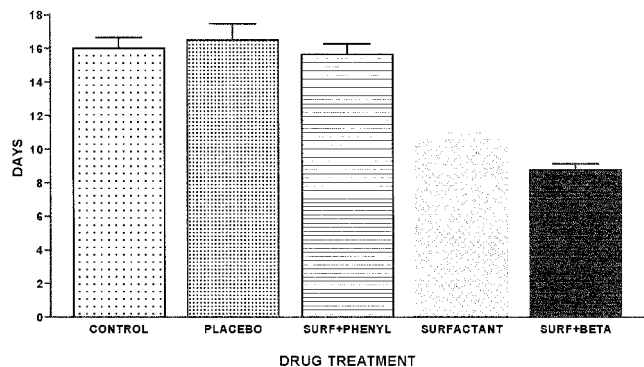


Fig. 1. Effect of intranasal metered dose inhaler (MDI) drugs on resolution of otitis media with effusion (OME) by otomicroscopy. Results for treatment with surfactant and surfactant plus betamethasone were statistically significant ($P < .05$).

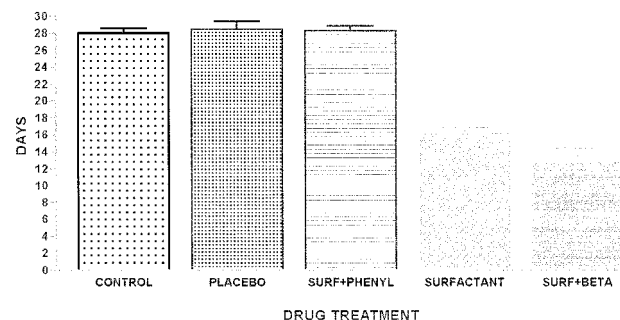


Fig. 2. Effect of intranasal MDI drugs on resolution of OME by tympanometry. Results for treatment with surfactant and surfactant plus betamethasone were statistically significant ($P < .05$).

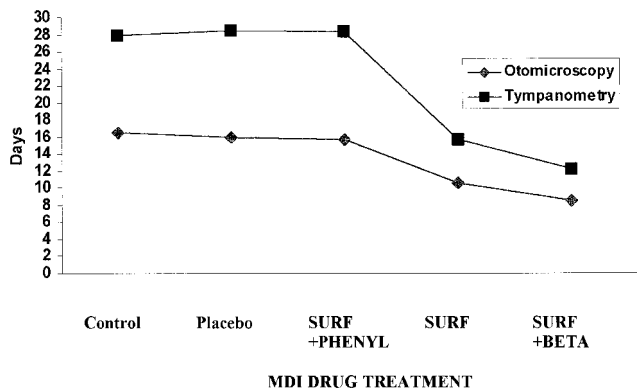


Fig. 3. Otomicroscopy versus tympanometry: days to OME resolution. The difference in days to normal between otomicroscopic evaluation and tympanometry evaluation was statistically significant ($P < .05$), but the curves are essentially parallel.

by otomicroscopy than by tympanometry: ears returned to normal appearance more promptly than they did to normal tympanic membrane or middle ear compliance.

CONCLUSION

The effectiveness of surfactant with and without the addition of steroid delivered intranasally via an MDI for the treatment of OME is clearly demonstrated in this gerbil study. Most interestingly, surfactant alone is efficacious in the treatment of OME. When a steroid is added, the period for resolution of effusion is significantly further reduced. Intranasal phenylephrine is not shown to be useful in the treatment of OME.

This animal study should be replicated in another cohort of gerbils to validate these findings. Nevertheless, based on the statistically significant results obtained in this gerbil study, we can make the following statement. The MDI aerosolized surfactant intranasal delivery system with and without steroid is practical, noninvasive, easily administered, delivers a concentrated drug dosage, and yields statistically significant results. Human trials are encouraged, as this drug and delivery device may be directly applicable to the pediatric population with OME.

ACKNOWLEDGMENT

The authors thank Hreday N. Sapru, PhD, Director of Neurological Sciences Laboratories at the University of Medicine and Dentistry or New Jersey–New Jersey Medical School, for his invaluable technical and scientific as-

sistance without which this project could not have taken place.

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