Lanthanum Inhibits Surfactant Secretion Stimulated by Lung Distension in Newborn Rabbits

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ABSTRACT. The effect of lanthanum on lung phospholipid secretion stimulated by lung distension was examined in 29.5-d gestation newborn rabbits. Lung lavage with saline alone produced 608 ± 109 μg phospholipid/lung (mean $\pm$ SEM), whereas lavage with 10^{-3} M lanthanum produced only 153 ± 40 μg phospholipid/lung ($p < 0.05$), the difference in yield being attributed to inhibited lavage-induced secretion with lanthanum. If this interpretation is correct, then an increased residual tissue surfactant should be found in lanthanum-lavaged lungs. Postlavaged lung was separated into IA, IB, and IC fractions by homogenization and then centrifugation with 0.25 and 0.65 M sucrose. The surfactant IB fraction was 763 ± 65 μg phospholipid/g wet lung for saline-lavaged lungs, but 1002 ± 90 μg phospholipid/g wet lung for lanthanum-lavaged lungs ($p < 0.002$). This confirms that decreased secretion with lanthanum was associated with increased residual surfactant in postlavaged lung. It is concluded that lanthanum is a potent inhibitor of surfactant secretion stimulated by lung distension. (*Pediatr Res* 30: 190–192, 1991)

The pulmonary surfactant is a lipoprotein complex synthesized and secreted by granular pneumocytes, and is necessary for stabilization of lung alveoli during exhalation. Although many factors may modulate secretion of surfactant, it is thought that lung distension is the principal stimulus (1). In newborn rabbits, we have suggested that increased intracellular calcium may be responsible for surfactant secretion induced by distension during lung lavage, because secretion is stimulated by increased calcium in the solution used to lavage the lungs (2). Previous work in our laboratory has demonstrated that lanthanum, an ion that blocks calcium entry into cells (3), inhibits the secretion of surfactant induced by lung lavage, the inhibition increasing with the concentration of lanthanum in the lavage fluid (4). Because the inhibition of secretion was large, the aim of our study was to confirm the inhibitory effect of lanthanum on surfactant secretion, by demonstrating a change in the residual tissue surfactant of postlavaged lung. We found that lanthanum produced both a decrease in lavage phospholipid and an increase in the tissue surfactant fraction.

MATERIALS AND METHODS

Preliminary experiments indicated that two litters of newborn rabbits would be required to demonstrate statistically significant differences for lavage fluid phospholipid between control and

experimental groups and that at least four litters would be required to demonstrate significant differences for tissue surfactant phospholipid.

The litters of seven pregnant New Zealand White rabbits (Ray Nichols Rabbitry, Beaumont, TX) were used in these experiments. Does of 29.5 d gestation, time of mating known to within 3 h, were anesthetized with methoxyflurane, and then pups were delivered in sequence by hysterotomy. After breathing room air for 30 min, the pups were killed with an injection of pentobarbital (20 mg). Each dead newborn rabbit was weighed, a tracheostomy tube was ligated in place, and the lungs were deflated by vacuum in preparation for lung lavage. Only the first six pups in each litter were used for these experiments, because only six samples could be processed at any one time.

In all experiments, the lungs of alternate pups were lavaged with physiologic saline containing 10^{-3} M lanthanum chloride, and the lungs of the other alternate pups were lavaged with saline alone. Thus, the experimental and control groups always consisted of littermate neighbor pairs, one experimental and one control.

The lavage procedure consisted of 10 successive washes. For each wash, fresh solution at room temperature was used; the volume instilled into the lung and immediately withdrawn was 1 mL/20 g body weight, estimated to represent two thirds of total lung capacity. Each wash during lavage took 1.5 min to complete, so the lavage procedure lasted 15 min. The volume of each pooled lavage sample was measured, and it was found that recovery was better than 90% for all samples.

The uncentrifuged lavage samples from the first two litters were analyzed for phospholipid after storage overnight at 4°C. For the measurement of phospholipid, lipids were extracted in chloroform-methanol, and phosphorus was assayed as previously described (5). Results were expressed as μg phospholipid/lung.

The tissue surfactant fraction of lavaged lung from all litters was analyzed using the method described by Frosolono (6). The lung was removed by dissection and blotted dry, and a piece of the left lower lobe (about 300 mg) was weighed. This sample was homogenized in 20 volumes of 0.25 M sucrose in 0.15 M NaCl, 0.01 M Tris-HCl, 0.001 M EDTA buffer (pH 7.4), using a Potter-Elvehjem homogenizer surrounded by packed ice to prevent overheating. Then an equal volume of 1.05 M sucrose in buffer was added to bring the concentration to 0.65 M sucrose. The percentage of sucrose was adjusted to exactly 22.4%, measured with a drop of solution in a hand-held refractometer (Fisher Scientific Co., Pittsburgh, PA). The material was then centrifuged in preweighed 17-mL nitrocellulose tubes at $48\,000 \times g$ for 60 min at 4°C (Beckman L5-50B ultracentrifuge), the pellet being the fraction designated as IC, and the supernate containing the fractions designated as IA and IB. A measured aliquot of the supernate was diluted with buffer to 0.25 M sucrose, the percentage of sucrose adjusted to exactly 8.6%. This preparation was vortexed and again centrifuged at $48\,000 \times g$ for 60 min at 4°C, the supernate being the IA fraction, and the pellet being the

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IB or tissue surfactant fraction. After decanting the supernate, the IB pellet was resuspended in 10 mL saline, 2 mL was extracted with 8 mL of 2:1 chloroform:methanol, and, after centrifugation at $1\,000 \times g$ at room temperature (Fisher Scientific, model no. 225), 1 mL of lower phase was assayed for phosphorus. Similarly, 2 mL of the IA supernate was extracted with chloroform:methanol and assayed for phosphorus. The results were expressed as μg phospholipid/g wet lung.

The nitrocellulose tube was weighed again, and the IC pellet resuspended in saline and transferred to a glass tube. After centrifugation at $1\,000 \times g$ at room temperature and collection of the supernate, the IC pellet was homogenized in 20 volumes of 2:1 chloroform:methanol, using a Polytron homogenizer (Brinkmann Instruments Co., Westbury, NY). The homogenate was passed through filter paper (Whatman No. 1), and the volume of filtrate was measured. Then 0.2 volumes of saline were added, and the mixture vortexed and centrifuged at $1\,000 \times g$ at 4°C (Beckman, model no. J2-21). After recording the volume of the lower phase, 0.1 mL was used in the phosphorus assay. In addition, the supernate from the above transfer process was also extracted with chloroform:methanol, and the amount in phosphorus assay was added to the IC fraction. The results were expressed as μg phospholipid/g wet lung.

Statistical analysis was performed using the Wilcoxon Signed Rank Sum Test (7). All data are reported as means $\pm$ SEM.

RESULTS

There was a large increase in the IB surfactant fraction of lavaged lung in those treated with lanthanum (Table 1), $1002 \pm 90 \mu\text{g}$ phospholipid/g wet lung, compared with $763 \pm 65 \mu\text{g}$ phospholipid/g wet lung in those treated with saline alone ($p < 0.002$). This suggests decreased secretion of tissue surfactant fraction during lavage with lanthanum. In addition, there was a significant increase in the IA fraction of those lavaged with lanthanum ($p < 0.05$).

In the two litters examined, the amount of phospholipid obtained by lavage with 10^{-3} M lanthanum was only one fourth that obtained by lavage with saline alone, a significant difference ($p < 0.05$), suggesting a reduction in lavage-associated phospholipid secretion (Table 2).

DISCUSSION

Phospholipid secretion produced by lung distension may be studied in the 29.5-d newborn rabbit model, because little of the yield from lavage represents the alveolar surfactant pool and

Table 1. *Surfactant (IB) and nonsurfactant (IA and IC) fractions of newborn rabbit lung tissue, after lavage with saline alone or 10^{-3} M lanthanum**

	n	Body wt (g)	Phospholipid ($\mu\text{g}/\text{g}$ wet lung)		
			IA	IB	IC
Saline	21	48.7 ± 1.9	598 ± 57	763 ± 65	4293 ± 368
Lanthanum	21	49.5 ± 1.7	$691 \pm 66^\dagger$	$1002 \pm 90^\ddagger$	4375 ± 278

* Values are means $\pm$ SEM. The data were obtained from seven litters.

$^\dagger p < 0.05$ compared with saline controls.

$^\ddagger p < 0.002$ compared with saline controls.

most of the yield represents fresh surfactant secretion induced by lavage (8). With lanthanum in the lavage solution, the phospholipid yield from lavage was only one fourth that with saline alone (Table 2), indicating a marked inhibition of lavage-induced surfactant secretion with lanthanum. This interpretation is confirmed by results for the tissue surfactant fraction (Table 1). If secretion is truly inhibited with lanthanum, there should be a significant increase in surfactant remaining in the lung tissue, as reflected in the IB fraction, after lavage with lanthanum. It was surprising to find also an increase in the IA fraction, which may mean that some surfactant was included in the IA fraction.

The procedure described by Frosolono (6) is a well-accepted method for isolating the surfactant fraction from lungs. The method used was that suggested for small samples; instead of collecting the IB fraction at the interface of a discontinuous sucrose gradient, the IB fraction was collected as a pellet after several centrifugations. The IA fraction, which floats in 0.25 M sucrose, contains mainly light membranous material. The IB fraction, which floats in 0.65 M sucrose, but sinks in 0.25 M sucrose, is known to be enriched with membranous material, some of it having the configuration of lamellar bodies, the storage form of surfactant. This fraction has been shown to be highly surface active, and is not found in tissues other than the lung (6). Material that is heavy enough to sink in 0.65 M sucrose, such as red cells and nuclei, is contained in the IC fraction. Neither the IA fraction nor the IC fraction is surface active.

The data in Table 2 do not give an exact estimate of the four fractions, IA, IB, IC, and lavage, because the units for the lavage fraction are different. However, inasmuch as the mean body weight was 51.6 g (Table 2) and 29.5-d rabbits have a lung weight/body weight ratio of 0.02 (9), it can be estimated that the mean lung weight was close to 1 g, in which case the data for lavage phospholipid/lung are a good estimate for lavage phospholipid/g lung. Allowing for this small inaccuracy, the mean total phospholipid per gram of lung was $5\,650 \mu\text{g}$ for lanthanum- and $5\,540 \mu\text{g}$ for saline-treated lungs (Table 2), values that agree closely. Despite this similarity in total lung phospholipid, it can be calculated that the distribution of the IB and lavage fractions was very different. The IB fraction for lanthanum-lavaged lungs represented 15% of total lung phospholipid, and the lavage fraction only 2.7%. This compared with 10.4% for the IB fraction, but 11% for the lavage fraction, in those lavaged with saline alone. This difference in distribution reflects active secretion with saline lavage, but inhibited secretion during lavage with lanthanum.

The present method of isolating the tissue surfactant fraction has been widely used in adult animals (10) and with *in vitro* systems (11). Rieutort *et al.* (12) used a similar isolation method in fetal rats, but examined nonlavaged lungs. They found that at 0.93 gestation the surfactant fraction phospholipid represented 16.8% of total lung phospholipid, and at 0.98 gestation it represented 25.6% of total lung phospholipid. In our rabbit experiments with saline controls at 0.95 gestation, the sum of the IB fraction and the lavage fraction phospholipid, an estimate of total surfactant fraction, was 21.4% of the total lung phospholipid, which is in reasonable agreement with results from the rat.

One further observation is that the sum of the IB and lavage fraction phospholipid in pups treated with lanthanum was only 17.7% of total lung phospholipid (Table 2), less than in saline-

Table 2. *Lung tissue and lung lavage fractions in newborn rabbits, lungs lavaged with saline alone or 10^{-3} M lanthanum**

	n	Body wt (g)	Lung phospholipid			
			IA	IB ($\mu\text{g}/\text{g}$ wet lung)	IC	Lavage ($\mu\text{g}/\text{lung}$)
Saline	6	48.7 ± 2.5	409 ± 40	578 ± 63	3945 ± 341	608 ± 109
Lanthanum	6	54.5 ± 3.3	481 ± 44	847 ± 107	4169 ± 302	$153 \pm 40^\dagger$

* Values are means $\pm$ SEM. The data were obtained from the first two litters of the seven litters reported in Table 1.

$^\dagger p < 0.05$ compared with saline controls.

treated controls. It is possible that the lung synthesized more surfactant during lavage with saline alone, because secretion was so active, or, alternatively, after treatment with lanthanum, some of the IB fraction was transferred to the IA or IC fractions. The latter interpretation is supported by the observation that in the lanthanum-treated pups there was a significant increase in the IA fraction (Table 1). This suggests that the increased IB fraction after lanthanum treatment may be an underestimate of the true magnitude of the change, and may explain why the increase in the IB fraction appeared to be less than the decrease in the lavage fraction after treatment with lanthanum (Table 2).

It is concluded that lanthanum inhibits surfactant secretion stimulated by lung distension during lavage. This was demonstrated by a reduction in lavage phospholipid and by an increase in the residual tissue surfactant phospholipid.

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