

# Lung Deposition of HFA Beclomethasone Dipropionate in an Animal Model of Bronchopulmonary Dysplasia

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**ABSTRACT:** The best delivery of a drug in ventilated neonates is obtained when using a small particle diameter solution administered *via* a spacer. Lung deposition of hydrofluoroalkane beclomethasone dipropionate (QVAR, 1.3  $\mu\text{m}$  particles), delivered *via* an Aerochamber-MV15, was measured in piglets under conditions mimicking ventilated severely ill neonates (uncuffed 2.5 mm endotracheal tube; peak pressure 16 cm  $\text{H}_2\text{O}$ ; respiratory rate 40/min). After determining the mass and particle size distribution of the  $^{99\text{m}}\text{Tc}$ -labeled and unlabeled drug, three lung deposition studies were performed: after 1 h of ventilation (controls,  $n = 18$ ), after 48 h aggressive ventilation inducing an acute lung injury (nine piglets out of the controls), and after increasing the pressure to 24 cm  $\text{H}_2\text{O}$  during drug delivery (five piglets out of the nine with acute lung injury). All piglets were then killed for lung histology. Results (median, range), expressed as a percentage of the delivered dose, were compared using an inferential or the Friedman test. While lung deposition was low, it was greater ( $p = 0.003$ ) in controls (2.66%, 0.50–7.70) than in piglets with histologically confirmed acute lung injury (0.26%, 0.06–1.28) or under a high-pressure ventilation (1.01%, 0.30–2.15). Lung deposition of QVAR in an animal model of ventilated neonates is low, variable, and dramatically affected by lung injury. (*Pediatr Res* 61: 21–25, 2007)

One of the most frequent complications in preterm newborns, particularly in low birth weight or extremely low birth weight preterm infants who survive prolonged mechanical ventilation for infant respiratory disease syndrome, is chronic lung disease. The exact mechanisms that cause fibroproliferative chronic lung disease remain unknown (1). The first step of this disease may be an acute lung injury corresponding to an end stage of severely immature or diseased lung experiencing oxidative stress, inflammation, and mechanical insult, with bronchial, alveolar, and capillary injuries and cell death (2). The inflammatory response to pulmonary insults, such as mechanical ventilation or supplemental oxygen, is presumed to be the most important factor (1–3).

This has been the rationale for the use of systemic corticosteroid therapy for the prevention or treatment of chronic lung disease in premature infants for more than two decades (4). Systemic administration of dexamethasone to mechanically ventilated preterm infants decreases the incidence of chronic lung disease and extubation failure but does not decrease overall mortality (5). However, the long-term risks of such a treatment are still a cause of concern, in particular with respect to brain development and lung growth in these preterm infants (1,5).

In a fashion similar to asthma treatment, inhaled corticosteroids have been administered to these patients in an attempt to reduce side effects. Most clinical studies using inhaled corticosteroids delivered *via* a spacer device or a nebulizer placed within the inspiratory circuit of a neonatal ventilator showed little or no effect on extubation rates and the incidence of chronic lung disease (5–9). This lack of effect may be due either to physiologic and/or anatomical problems (small airways, low tidal volume, etc.) or to technical factors limiting aerosol efficacy in intubated and ventilated preterm infants (small endotracheal tube, large particles diameter, etc.) (10). It may also be due to the lung disease itself, but this has never been explored.

*In vitro* studies suggest that maximal delivery of an inhaled corticosteroid through a narrow endotracheal tube is obtained from a drug in solution administered through a spacer device placed between the Y-piece and the endotracheal tube (11–14). Moreover, inasmuch as conventional aerosols with a MMAD of 3–4  $\mu\text{m}$  appear to penetrate poorly through small 2.5- or 3.0-mm-diameter endotracheal tubes, it has been proposed that the use of smaller particle size aerosols may be more efficacious (15). The development of new inhaled corticosteroids in solution, with a 3-fold smaller MMAD, has improved lung delivery in infants (16) and may possibly be useful in such a neonatal situation. However, to the best of our knowledge, no study has been published in the medical literature regarding the lung deposition obtained with these new corticosteroids delivered *via* a spacer device in ventilated neonates.

The aim of the present study was thus to characterize the deposition of radiolabeled QVAR [a solution of 134a-

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Abbreviation: MMAD, mass median aerodynamic diameter

hydrofluoroalkane beclomethasone dipropionate characterized by a high amount of small particles approximately 1  $\mu\text{m}$  diameter (16,17), 100  $\mu\text{g}$  per puff, 3M Santé Laboratories, Cergy Pontoise, France], administered *via* an Aerochamber-MV15 (Trudell Medical International, London, ON, Canada) in three groups of intubated and ventilated individuals: 1) controls, 2) individuals having an acute lung injury, and 3) individuals having an acute lung injury and an increased peak inspiratory pressure during the drug delivery in an attempt to increase aerosol deposition by increasing tidal volume. Because radiolabeled studies are deemed unethical in preterm infants, a piglet model was used and lung deposition of QVAR was studied before and after inducing an acute lung injury through 48 h aggressive mechanical ventilation (18,19).

## MATERIALS AND METHODS

### *In Vitro* Study

**Aerosol characteristics.** The particle size distribution of QVAR was measured using the Andersen cascade impactor (series 20-800 Mark II, Ecomesure, Janvry, France) operating at 28.3 L/min. The endotracheal tube was connected directly to the top of the impactor. The impactor pump was turned on, and 10 puffs were delivered at the input of the trachea. Each plate of the impactor was introduced in a container containing 20 mL of 99.6% pure methanol. The upper part of the impactor, the so-called trachea, was also rinsed with 20 mL of methanol. For each plate, the concentration of beclomethasone dipropionate was determined by UV-spectrophotometry (238 nm). Two QVAR canisters were used, each one for three experiments, *i.e.* a total of six experiments were performed. MMAD were also calculated for each distribution.

**<sup>99m</sup>Tc labeling of QVAR metered-dose inhaler.** Labeling of the content of the QVAR metered-dose inhaler was performed using pertechnetate (<sup>99m</sup>Tc). <sup>99m</sup>Tc was dissolved in 0.5 mL normal saline, placed into a syringe, and calibrated to 555 MBq per syringe. The QVAR canister was frozen at  $-196^{\circ}\text{C}$  in liquid nitrogen (10 min). A small hole was pierced at the bottom of the canister using a drill. <sup>99m</sup>Tc was introduced into the canister using a syringe equipped with a needle. Before and after the introduction of <sup>99m</sup>Tc into the canister, the radioactivity contained within the syringe was measured in an activimeter (Capintec Inc., CRC Ariès, France). Finally, the hole was hermetically sealed by a piece of rubber firmly maintained on the canister using a metallic collar.

The particle size distribution of labeled QVAR was measured according to the methods described above regarding unlabeled QVAR (two labeled canisters for  $2 \times 3$  experiments), except that for each plate the activity was also measured in an activimeter (ISOCOMP I). For each measurement done with the labeled QVAR, the activity and the mass of beclomethasone dipropionate were compared to determine the beclomethasone dipropionate mass/<sup>99m</sup>Tc activity relationship. Particle size distributions were compared in terms of beclomethasone dipropionate mass and <sup>99m</sup>Tc activity for the labeled QVAR, and in terms of beclomethasone dipropionate mass for the unlabeled and labeled beclomethasone dipropionate. MMAD were also calculated for each distribution.

### *In Vivo* Study

**Animal model: general design.** Lung deposition of QVAR was studied in 18 2-d-old Meisham males piglets ( $1.7 \pm 0.3$  kg; range, 1.22–2.40 kg). Three different lung deposition studies were performed: after 1 h of ventilation (controls), after 48 h of aggressive ventilation inducing an acute lung injury but in the same respiratory conditions as controls (ALI group), and after a 50% increase in peak inspiratory pressure during drug delivery in an attempt to increase aerosol deposition by increasing tidal volume (ALI-hiPIP group). Based on previous experiments (20), the number of piglets was chosen to obtain at least four piglets in the third group. We initially included 20 piglets, but 2 died during the first experiment. A total of 18 piglets underwent the first experiment (controls,  $n = 18$ ). Half of the animals were killed for lung histology and the other half underwent the second experiment (ALI,  $n = 9$ ). Four of these nine piglets were killed and five completed the third phase of the studies (ALI-hiPIP,  $n = 5$ ). The last five piglets were also killed to verify that they had a similar histologic lung injury score to piglets in the ALI group. The

study was approved by the Animal Research Ethics Committee of Tours University.

**Controls.** The control ( $n = 18$ ) piglets were anesthetized with an intraperitoneal injection of pentobarbital, 25 mg/kg, and continuous isoflurane (Forane) inhalation through an adapted face mask. Animals were intubated with an uncuffed Portex 2.5 mm endotracheal tube, the extremity of which was placed 11 cm from the level of the teeth. All the animals were ventilated for one hour with a Servo 900C ventilator (Siemens Medical Solutions USA, Inc., Malvern, PA) through a neonatal circuit before the delivery of <sup>99m</sup>Tc-QVAR. The ventilator parameters were chosen after consulting four different neonatologists working in neonatal intensive care units for mimicking ventilation levels of neonates suspected to present an acute lung injury and to evaluate to a subsequent chronic lung disease. These settings were as follows: peak inspiratory pressure, 16 cm H<sub>2</sub>O; positive end-expiratory pressure, 4 cm H<sub>2</sub>O; respiratory rate, 40/min; inspiratory time, 0.5 s; and inhaled fraction in oxygen, 0.4. After a 1-h exposition to this level of ventilation, <sup>99m</sup>Tc QVAR deposition was studied. After which, 9 of the 18 piglets were killed for lung histology.

**ALI group.** The nine remaining piglets (the ALI group) were aggressively ventilated for 48 h based on protocols reported by Davis *et al.* (18) and Easa *et al.* (19) who have described a model of ventilation for inducing acute lung injury. The positive end-expiratory pressure was 2 cm H<sub>2</sub>O, respiratory rate 50/min, inspiratory time 0.4 s, and inhaled fraction in oxygen 1. Peak inspiratory pressure was adjusted individually with the aim of maintaining PaCO<sub>2</sub> (determined invasively every 6 h by arterial puncture after tracheal aspiration) between 15 and 20 mm Hg. Oxygen was humidified through an artificial humidification device. A catheter was placed into the jugular vein and a continuous infusion of a solution of 10% glucose (80 mL/kg/d) was initiated. Sedation was maintained by the continuous infusion of pentobarbital, 7.5 mg/kg/h. The carotid artery was dissected for blood analyses.

The piglets were placed into an incubator and their rectal temperature, which had to be physiologically maintained at  $38.5^{\circ} \pm 0.5^{\circ}\text{C}$ , was controlled every 3 h. Antibiotics (ampicillin, 50 mg/kg/d, and gentamicin, 2.5 mg/kg/d) were administered by the intramuscular route twice a day. The piglets were turned on their left and right side alternately every 3 h for alleviating possible atelectasis.

After 48 h, these nine piglets underwent a second experiment with <sup>99m</sup>Tc-QVAR. Before drug delivery, they were ventilated for 1 h using ventilator parameters that were identical to the first experiment. Tracheal aspiration was performed 15 min before <sup>99m</sup>Tc-QVAR administration to ensure that no endotracheal tube occlusion was present.

Four of these animals were killed at the end of this second experiment for lung histology.

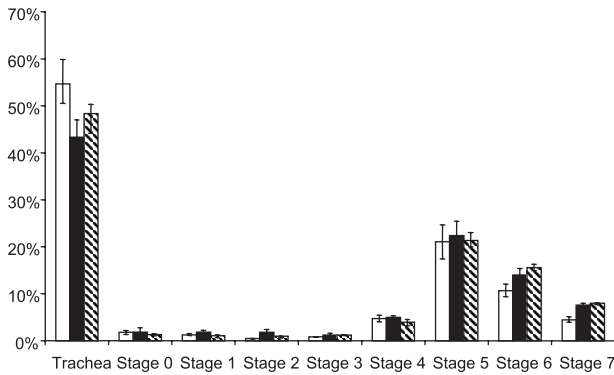
**ALI-hiPIP group.** The five remaining piglets (the ALI-hiPIP group) underwent a third experiment, 30 min after the second phase. The ventilator settings were modified by increasing peak inspiratory pressure to 24 cm H<sub>2</sub>O, *i.e.* 50% higher than the peak inspiratory pressure used during the first and second experiments. At the end of this third experiment, the five piglets were also killed for lung histology.

**<sup>99m</sup>Tc-QVAR delivery and deposition study.** Ten separated puffs of <sup>99m</sup>Tc-QVAR were administered through a 145-mL spacer device (Aerochamber-MV15, Trudell Medical International) placed between the Y-piece and the Portex 2.5 mm internal diameter endotracheal tube. Each puff was delivered manually during the inspiratory cycle, the animals being placed in the supine position. Importantly, each puff was given separately, after an interval of five respiratory cycles.

For each experiment, the delivered dose was determined by the measurement of the pre- and post-administration radioactivity of the canister placed into a dose calibrator (Capintec Inc., CRC Ariès, France). Immediately after the administration of 10 puffs, the piglets were scanned during 60 s using an Orbiter 75 gamma camera (matrix  $128 \times 128$ , Siemens Medical Solutions USA, Inc.) connected to a PC computer. The amount of radioactivity deposited in the right and left lungs was determined by drawing regions of interest from the digitalized images corrected by tissue attenuation coefficients derived from phantoms with pertechnetate mimicking the piglets. Lung dose was reported as a percentage of the delivered dose given for that experiment.

The five animals of the ALI-hiPIP group underwent two experiments at 30 min intervals. In these piglets, scintigraphic deposition was calculated by the difference between the deposition values obtained the same day at the end of the third experiment (24 cm H<sub>2</sub>O peak inspiratory pressure) and during the second experiment (16 cm H<sub>2</sub>O peak inspiratory pressure).

**Lung histology.** At the end of the study, all the animals were killed with a lethal dose of intravenous pentobarbital. A bilateral thoracotomy was performed and, after careful dissection, the trachea and lungs were extracted. The trachea was then catheterized with a small smooth nozzle. A syringe containing 50 mL formaldehyde was connected to the nozzle and the content was slowly administered under constant pressure until the lungs were correctly inflated and the liquid exiting from lungs was clear, without blood. The



**Figure 1.** Deposition fractions on each impactor stage expressed as percent total deposition, in terms of activity (□) and mass (■) for the labeled QVAR and in terms of mass (▨) for the unlabeled QVAR (expressed in medians; error bars are quartiles).

lungs were then placed into formaldehyde solution for at least 48 h to allow for the decay of the technetium. After fixation, the end of the trachea and each lung lobe were sectioned in three different areas for light microscopy (each piece was cut into two equal pieces that were also cut in two further equal sections, the inferior part of which constituting the three areas of observation). These sections were embedded in paraplast and stained with trichrome. The pathologist was blinded as to the specific treatment of the individual piglet. Abnormalities in inflation patterns, hemorrhage, interstitial edema, intralveolar exudates, and acute inflammation were examined. Changes were graded from 0 (the least) to 4 (the most abnormal), based on the assessment of degree and severity of involvement (maximal score of 20) (18,19).

**Statistical analysis.** The statistical analysis was performed using StatXact statistical software (Cytel Software Corp., Cambridge, MA). For the *in vitro* study, beclomethasone dipropionate mass measurements and  $^{99m}\text{Tc}$  activities were correlated by linear regression, this correlation being characterized using the Pearson's correlation exact test. This test was also used to correlate mass distribution for labeled and unlabeled QVAR. MMAD values were expressed as mean  $\pm$  SEM. Deposition fractions on the impactor stages were expressed in medians and the ranges in quartiles. To compare deposition data between the three groups of piglets the nonparametric statistical Friedman test was used. For all statistical tests, a  $p$  value  $\leq 0.05$  was considered statistically significant.

## RESULTS

### *In Vitro* Data

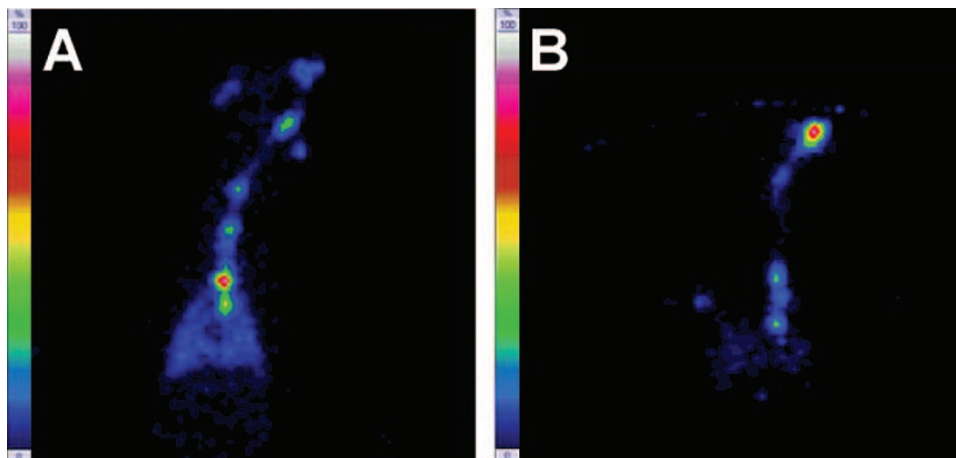
A high quantity of beclomethasone was delivered from the QVAR canister *via* the Aerochamber-MV15, using the ventilator settings described above and an uncuffed 2.5 mm Portex tube; at the distal tip of the endotracheal tube, this amounted to  $22.02 \pm 1.67\%$  of the nominal dose.

The comparison of the beclomethasone dipropionate mass and  $^{99m}\text{Tc}$  activity measurements [ $n = 54$ , six measurements per plate ( $n = 8$ ) and trachea] showed a very significant 99% correlation ( $p < 0.0001$ ). The beclomethasone dipropionate mass ( $y$ )/ $^{99m}\text{Tc}$  activity ( $x$ ) relationship ( $y = 1.027x - 0.0111$ ,  $R^2 = 0.94$ ) indicated that  $^{99m}\text{Tc}$  activity closely followed the actual mass of beclomethasone dipropionate. Particle size distributions according to beclomethasone dipropionate mass measurements for labeled and unlabeled QVAR ( $n = 54$ ) showed a very significant 99% correlation ( $p < 0.0001$ ). Figure 1 shows the deposition fractions on each impactor stage normalized to the total amount deposited, in terms of activity and mass regarding the labeled QVAR and in terms of mass regarding the unlabeled QVAR (expressed in medians and quartiles). For labeled QVAR, the MMAD was  $1.4 \pm 0.1 \mu\text{m}$  in terms of activity and  $1.4 \pm 0.1 \mu\text{m}$  in terms of beclomethasone dipropionate mass. For unlabeled QVAR, the MMAD was  $1.3 \pm 0.1 \mu\text{m}$  in terms of beclomethasone dipropionate mass.

### *In Vivo* Data

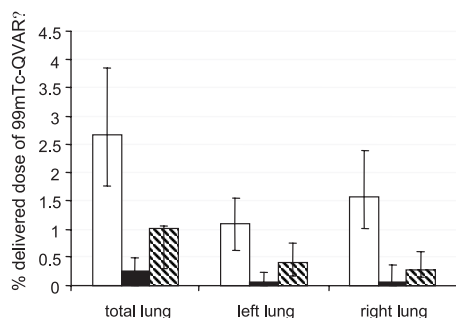
***Tc99m-QVAR deposition.*** All the 18 piglets completed the experiments. The nine piglets in the ALI group who underwent the 2 d of aggressive ventilation were all able to participate in the second lung deposition study. Incidents during these 2 d were as follows: accidental extubation of short duration due to decreased sedation ( $n = 2$ ), occlusion of infusion lines ( $n = 3$ ), occluded carotid catheter ( $n = 1$ ). The  $\text{PaCO}_2$  of these piglets was maintained at  $17.4 \pm 2.1$  mm Hg. This required inspiratory pressures ranging from 34 to 48 cm  $\text{H}_2\text{O}$ . An example of scintigraphic images obtained from the same piglet before and after the 2 d of aggressive ventilation is shown in Figure 2.

The median delivered activity per puff of  $^{99m}\text{Tc}$ -QVAR was 165  $\mu\text{Ci}$ . Results of lung deposition are presented in Figure 3. Total lung deposition in the 18 control piglets ranged from 0.50 to 7.70% (median, 2.66%) of the delivered dose. The second study, also conducted with a peak inspiratory pressure of 16 cm  $\text{H}_2\text{O}$ , in previously aggressively ventilated piglets (ALI group), showed a dramatic decrease in lung deposition in comparison to controls, with a median total lung



**Figure 2.** Typical examples of scintigraphic images showing  $^{99m}\text{Tc}$ -QVAR lung deposition in the same neonatal piglet before (A) and after inducing an acute lung injury (B) by 2 d of aggressive ventilation (delivery of drug was performed in a piglet intubated with an uncuffed 2.5 mm Portex ID endotracheal tube and ventilated with a Servo 900C adjusted for peak inspiratory pressure, 16 cm  $\text{H}_2\text{O}$ ; peak end-expiratory pressure, 4 cm  $\text{H}_2\text{O}$ ; respiratory rate, 40/min; inspiratory time, 0.5 s; and inhaled fraction in oxygen, 0.4).





**Figure 3.** Median lung deposition of  $^{99m}\text{Tc}$ -QVAR in intubated and ventilated neonatal piglets with different lung and mechanical ventilation status at the time of drug administration (results are expressed as percentage of the delivered dose and compared between groups by the nonparametric Friedman test). A statistical difference was found between the three groups for total lung, right lung, and left lung deposition;  $p = 0.003$ ,  $0.003$ , and  $0.05$ , respectively). Eighteen control piglets (□) were ventilated with a Servo 900C adjusted for peak inspiratory pressure, 16 cm  $\text{H}_2\text{O}$ ; positive end-expiratory pressure, 4 cm  $\text{H}_2\text{O}$ ; respiratory rate, 40/min; inspiratory time, 0.5 s; and inhaled fraction in oxygen, 0.4. Nine piglets (■), acute lung injury group taken from the control group were aggressively ventilated for 2 d before undergoing a new  $^{99m}\text{Tc}$ -QVAR delivery study under the same ventilation conditions as previously described. ALI-hiPIP (▨) piglets included five of the previous nine piglets, who underwent a third  $^{99m}\text{Tc}$ -QVAR delivery study with a peak inspiratory pressure of 24 cm  $\text{H}_2\text{O}$ .

deposition of 0.26% (range, 0.06–1.28%;  $p = 0.0001$ ). In the five piglets submitted to the third deposition study (ALI-hiPIP group), increasing peak inspiratory pressure to 24 cm  $\text{H}_2\text{O}$  resulted in increasing the median lung deposition to 1.01% (range, 0.30–2.15%) of the delivered dose (Fig. 3). However, the total lung deposition remained higher in controls compared with the ALI and ALI-hiPIP groups ( $p = 0.003$ ). The same characteristics were noted when considering the right and left lung deposition in each group instead of total lung deposition (Fig. 3).

**Lung histology.** All nine killed controls had normal tracheal and lung histology (score from 0 to 1.1). All four piglets from the ALI group and the five piglets from the ALI-hiPIP group showed tracheal lesions such as necrosis with epithelial shedding, mucosal, and glandular inflammation. Necrosis and inflammation of segmental bronchial were also reported. In these nine aggressively ventilated piglets, inflation, alveolar exudate, interstitial edema, hemorrhage, congestion, and inflammation were found at variable degrees within different areas of the lungs. Overall, lesions were prominent in the lower lung lobes, but some degree of acute lung injury was demonstrable in all the lung sections examined. The histologic score varied from 6.8 to 12.7 within piglets, with no difference between piglets from the ALI and ALI-hiPIP groups. There was no correlation between median total lung deposition and the histologic score in piglets with acute lung injury.

## DISCUSSION

Little is known about lung deposition of inhaled drugs in ventilated neonates. Lung deposition, principally estimated by pharmacokinetic studies, is considered to be low (10). To the best of our knowledge, because of ethical and technical reasons, no radioactive quantification of lung deposition with

inhaled steroids exists in such patients. This is the first study that quantifies lung deposition of inhaled steroids solution with a small particle diameter in an animal model comprising neonatal piglets, and which compares aerosol deposition in normal and severely and acutely injured lungs. Lung deposition of  $^{99m}\text{Tc}$ -labeled 134a-hydrofluoroalkane beclomethasone dipropionate administered through a spacer device is extremely low and variable in neonatal piglets intubated with an uncuffed 2.5 mm endotracheal tube and ventilated using neonatal parameters. When inducing acute lung injury by 2 d of aggressive artificial ventilation, a 10-fold decrease in lung deposition is observed. Increasing the peak inspiratory pressure from 16 to 24 cm  $\text{H}_2\text{O}$  during drug delivery in piglets with an acute lung injury improves lung deposition, but values obtained remain lower than in control animals.

Our results show low lung deposition of inhaled steroids and a high variability in lung deposition between individuals. This intersubject variability may be due to the experimental procedure in which tracheal aspiration preceding drug delivery could have induced a bronchospasm. However, we believe that the manual mode of drug delivery, as usually applied in clinical practice, is the most likely explanation. These findings strongly argue in favor of the development of an automatic breath-synchronized system of drug delivery in mechanically ventilated patients.

In our study, aggressively ventilated piglets have histologically proven acute lung injury. One can presume that these animals with lung damages are a good model for ventilated neonates susceptible to be treated with inhaled steroids. In such piglets the median lung deposition was 10-fold less than in controls with histologically normal lungs. Assuming that inhaled steroids are useful in such clinical situations, the reported clinical inefficacy of inhaled steroids may be explained by such very poor lung deposition. For instance, a recent study (9) concluded that inhaled steroids (100  $\mu\text{g}/\text{kg}/\text{d}$ ), delivered from a pressurized metered-dose inhaler connected through an adapter placed between a 250-mL Laerdal resuscitation bag and a 55.3  $\text{cm}^3$  spacer connected directly to the uncuffed endotracheal tube, had no effect in intubated infants. Interestingly, the combined fact that the investigators (9) used a classical pressurized metered-dose inhaler of beclomethasone dipropionate suspension (which generates particles of 3–4  $\mu\text{m}$  diameter), and that only a very reduced amount of drug exited the distal tip of the endotracheal tube (3% of the delivered drug, *i.e.* 2.40–3.69  $\mu\text{g}/\text{kg}/\text{d}$  delivered to lungs) likely explains their results, given that in the present study, as much as 22% of the drug solution exiting the distal tip of the endotracheal tube resulted in no more than 0.26% lung deposition with extrafine particles. One of the possible solutions with such a low lung deposition would be to increase the dose of inhaled steroids. Using 10-fold higher doses of fluticasone propionate (21) or beclomethasone dipropionate (22) than in the Rozycki's study (9) induces a positive clinical effect. Unfortunately, at such doses, side effects were also observed (21).

The first hypothesis proposed to explain the major decrease in lung deposition in piglets with acute lung injury is that the major structural lungs modifications, compared with controls, alter deposition. Lung infection may also play a role, but our

piglets were treated by antibiotics and no infectious lesions were identified postmortem. We did not measure the tidal volume of the piglets during the study, but the observed lesions were probably responsible for a decreased dynamic lung compliance and tidal volume. Consequently, decreased inhalation and modified lung distribution of the drug, even with a particle MMAD as small as what is generated by QVAR may have occurred. Decreased tidal volume is unlikely to be the sole explanation, inasmuch as a 50% increase in peak inspiratory pressure only partially restored lung deposition in severely injured piglets lungs. Moreover, this increase in pressure to increase tidal volume cannot be recommended in clinical practice because of additional barotrauma risk. A combination of decreased tidal volume with more flow turbulence and/or more air leaks around the endotracheal tube may be considered. Further studies, particularly using other modes of ventilation than pressure-controlled ventilation as in our study, should be conducted to evaluate the importance of the ventilatory volumes on deposition of inhaled steroids into lungs with acute injury.

Our study, based on results of filter deposition (11–14), theoretically exploring the best conditions to administer an inhaled drug *via* a spacer device, irrespective of its type, placed within the inspiratory limb of the neonatal ventilator circuit, has been disappointing. Other delivery systems have to be considered, particularly nebulizers, which enable delivery of high doses of drugs. For instance, a dosimetric jet nebulizer used for delivering twice daily 500  $\mu\text{g}$  budesonide to very low birth weight infants requiring mechanical ventilation (median gestational age, 26 wk, and birthweight, 805 g) increases the rate of extubation (23). Therefore, the most promising nebulizers are probably the membrane-vibrating nebulizers that allow a mean lung deposition as high as 16% in intubated and ventilated 2.6-kg macaque monkeys (20). However, lung delivery obtained with these nebulizers should be compared with what can be obtained through direct instillation of the drug into the endotracheal tube. Indeed, such an instillation of budesonide has been reported to deliver 14–28% of the dose in the large airways, and 4–11% in alveolar and peripheral areas in ventilated rabbits (24).

In conclusion, lung deposition of small-particle diameter inhaled steroids delivered *via* a spacer device in piglets mimicking intubated and ventilated neonatal infants is low and highly variable. This deposition is even lower when an acute lung injury is induced by 48 h of aggressive ventilation. The inefficacy of inhaled steroids in neonates requiring mechanical ventilation cannot be affirmed until further lung deposition

studies with improved devices in acute lung injury are carefully conducted.

## REFERENCES

1. Bolt RJ, van Weissenbruch MM, Lafeber HN, Delemarre-van de Waal HA 2001 Glucocorticoids and lung development in the fetus and preterm infant. *Pediatr Pulmonol* 32:76–91
2. Zoban P, Cerny M 2003 Immature lung and acute lung injury. *Physiol Res* 52:507–516
3. Speer CP 2003 Inflammation and bronchopulmonary dysplasia. *Semin Neonatol* 8:29–38
4. Mammel MC, Green TP, Johnson DE, Thompson TR 1983 Controlled trial of dexamethasone therapy in infants with bronchopulmonary dysplasia. *Lancet* 1:1356–1358
5. American Academy of Pediatrics Committee on Fetus and Newborn 2002 Postnatal corticosteroids to treat or prevent chronic lung disease in preterm infants. *Pediatrics* 109:330–338
6. Lister P, Iles R, Shaw B, Ducharme F 2000 Inhaled steroids for neonatal chronic lung disease. *Cochrane Database Syst Rev* 2000;(3):CD002311
7. Shah SS, Ohlsson A, Halliday H, Shah VS 2003 Inhaled versus systemic corticosteroids for the treatment of chronic lung disease in ventilated very low birth weight preterm infants. *Cochrane Database Syst Rev* 2003;(2):CD002057
8. Shah SS, Ohlsson A, Halliday H, Shah VS 2003 Inhaled versus systemic corticosteroids for preventing chronic lung disease in ventilated very low birth weight preterm neonates. *Cochrane Database Syst Rev* 2003;(1):CD002058
9. Rozycki HJ, Byron PR, Elliott GR, Carroll T, Gutcher GR 2003 Randomized controlled trial of three different doses of aerosol beclomethasone versus systemic dexamethasone to promote extubation in ventilated premature infants. *Pediatr Pulmonol* 35:375–383
10. Cole CH 2000 Special problems in aerosol delivery: neonatal and pediatric considerations. *Respir Care* 45:646–651
11. Rozycki HJ, Byron PR, Dailey K, Gutcher GR 1991 Evaluation of a system for the delivery of inhaled beclomethasone dipropionate to intubated babies. *Dev Pharmacol Ther* 16:65–70
12. Arnon S, Grigg J, Nikander K, Silverman S 1992 Delivery of micronized budesonide suspension by metered dose inhaler and jet nebulizer into a neonatal ventilator circuit. *Pediatr Pulmonol* 13:172–175
13. Pelkonen AS, Nikander K, Turpeinen M 1997 Jet nebulization of budesonide suspension into a neonatal ventilator circuit: synchronized versus continuous nebulizer flow. *Pediatr Pulmonol* 24:282–286
14. Turpeinen M, Nikander K 2001 Nebulization of a suspension of budesonide and a solution of terbutaline into a neonatal ventilator circuit. *Respir Care* 46:43–48
15. Ahrens RC, Ries RA, Pependorf W, Wiese JA 1986 The delivery of therapeutic aerosols through endotracheal tubes. *Pediatr Pulmonol* 2:19–26
16. Janssens HM, De Jongste JC, Hop WC, Tiddens HA 2003 Extra-fine particles improve lung delivery of inhaled steroids in infants: a study in upper airway model. *Chest* 123:2083–2088
17. Leach CL, Davidson PJ, Boudreau RJ 1998 Improved airway targeting with the CFC-free HFA-beclomethasone metered-dose inhaler compared with CFC-beclomethasone. *Eur Respir J* 12:1346–1353
18. Davis JM, Dickerson B, Metlay L, Penney DP 1991 Differential effects of oxygen and barotrauma on lung injury in the neonatal piglet. *Pediatr Pulmonol* 10:157–163
19. Easa D, Finn KC, Balaraman V, Sood S, Wilkerson S, Takenaka W, Mundie TG 1995 Preservation of pulmonary function in the ventilated neonatal piglet with normal lungs. *Pediatr Pulmonol* 19:174–181
20. Dubus JC, Vecellio L, De Monte M, Fink JB, Grimbert D, Montharu J, Valat C, Behan N, Diot P 2005 Aerosol deposition in neonatal ventilation. *Pediatr Res* 58:10–14
21. Fok TF, Lam K, Dolovich M, Ng PC, Wong W, Cheung KL, So KW 1999 Randomised controlled study of early use of inhaled corticosteroid in preterm infants with respiratory distress syndrome. *Arch Dis Child Fetal Neonatal Ed* 80:F203–F208
22. Giep T, Raibble P, Zuerlein T, Schwartz ID 1996 Trial of beclomethasone dipropionate by metered-dose inhaler in ventilator-dependent neonates less than 1500 grams. *Am J Perinatol* 13:5–9
23. Jonsson B, Eriksson M, Soder O, Broberger U, Lagererantz H 2000 Budesonide delivered by dosimetric jet nebulization to preterm very low birthweight infants at high risk for development of chronic lung disease. *Acta Paediatr* 89:1449–1455
24. Fajardo C, Levin D, Garcia M, Abrams D, Adamson I 1998 Surfactant versus saline as vehicle for corticosteroids delivery to the lungs of ventilated rabbits. *Pediatr Res* 43:542–547