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Antibiotic Prophylaxis Improves *Ureaplasma*-Associated Lung Disease in Suckling Mice

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ABSTRACT: *Ureaplasma* infection is associated with increased lung disease in high-risk neonates. Our goal was to determine the impact of antibiotic prophylaxis on *Ureaplasma* and oxygen-induced lung disease in newborn mice. In animal model development and prophylaxis experiments, pups were randomly assigned to either 0.8 or 0.21 inspired oxygen concentration [fraction of inspired oxygen (FiO_2)] from 1 to 14 d of age and either *Ureaplasma* or 10 B media daily from 1 to 3 d. All pups were observed for growth and survival. Surviving pups had culture and PCR evaluated for blood, bronchoalveolar lavage, and lung, and lung weights, pathology, morphometry, histology, and immunohistochemistry were determined. In prophylaxis experiments, erythromycin, azithromycin, or normal saline was given for the first 3 d, and minimum inhibitory concentration and pharmacokinetics were determined. In model development, 0.8 FiO_2 and *Ureaplasma* infection survival and growth were significantly decreased and lung edema and inflammation were significantly increased. In prophylaxis experiments, we observed significantly improved survival and growth with azithromycin *versus* normal saline controls, whereas erythromycin was not significantly different from controls, and decreased inflammatory response with azithromycin *versus* normal saline and erythromycin. In a neonatal mouse model of *Ureaplasma* and oxygen-induced lung disease, appropriate antibiotic prophylaxis improves survival and morbidity and decreases lung inflammation. **(*Pediatr Res* 66: 197–202, 2009)**

Ureaplasma, commonly isolated from the newborn lower respiratory tract (1–3), is insensitive to beta-lactam antibiotics (4). This organism can be found in the genital tract of pregnant women. Amniotic fluid or placenta infection with *Ureaplasma* is associated with preterm labor, prematurity, low birth weight, and fetal death (1,2,5,6).

Premature infants with *Ureaplasma* infection have an increased incidence of pneumonia and bronchopulmonary dysplasia (BPD) (1,5). *Ureaplasma* lung infection is associated with increased polymorphonuclear leukocytes in tracheal aspirates and focal loss of ciliated epithelium (5,6) and is more likely to progress to dysplastic changes and chronic lung disease compared with pneumonia attributed to other bacteria (4).

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Erythromycin has been used to treat or prevent *Ureaplasma*-associated lung disease due to unacceptable side effects of other potential agents (7,8). A Cochrane review of 17 studies evaluating erythromycin for the prevention of chronic lung disease found no decrease in BPD incidence or severity (8). In another study, erythromycin failed to eradicate colonization or infection in up to 50% of patients (7). However, these studies did not evaluate organism antibiotic sensitivity or antibiotic kinetics. In addition, the effectiveness of antibiotic prophylaxis or treatment in an animal model of *Ureaplasma*-associated lung disease has not been evaluated. Thus, we developed a neonatal mouse model of *Ureaplasma*-associated lung disease and investigated the effectiveness of antibiotic prophylaxis in reducing mortality and morbidity.

METHODS

Organism. A clinical *Ureaplasma* strain (serotype 14, *Ureaplasma parvum*) isolated from a 25-wk gestation placenta was diluted 1/100 with 10B broth (Remel Inc., Lenexa, KS) and grown before each use from a frozen stock solution (5×10^6 color changing units/mL). The *U. parvum* species was selected because it is most commonly isolated from clinical specimens (2). Twenty microliters of the resultant solution was added to 50 mL of 10B broth and grown for 16 h at 32.5°C, centrifuged at 3000 rpm for 30 min, and the pellets resuspended in 900 μL of 10B. This final solution used for infection had a concentration of 5×10^6 color changing units/mL.

Animals. FVB albino mice (Charles River Laboratories Inc., Wilmington, MA) were time impregnated and fed antibiotic free water and food *ad libitum*. Pups were kept with dams throughout each experiment. At 14 d, surviving pups were euthanized with injection of 2.25–4.5 mL/kg of 20% Rodent Comboanesthetic III (ketamine 37.6 mg/mL, xylazine 1.92 mg/mL, and acepromazine 0.38 mg/mL). The Institutional Animal Use Committee approved the experiments.

Minimum inhibitory concentration and minimum bactericidal concentration. A microdilution method was performed as described previously to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (9). Culture confirmation was performed on an A7 Agar plate (9).

Animal model. One-day-old littermates randomly received either 0.21 or 0.8 inspired oxygen concentration [fraction of inspired oxygen (FiO_2)] (10,11). Four hours later, pups randomly received either *Ureaplasma* or 10B broth (see Organism section above) intramuscularly as follows: 0.1 mL on d 1, 0.2 mL on d 2, and 0.3 mL on d 3. Dams were alternated every 24 h between 0.21 and 0.8 FiO_2. A 0.8-FiO_2 environment resulted from mixing compressed air and oxygen (Bird low flow oxygen blender, Palm Springs, CA) in a Plexiglas chamber. Carbon dioxide was collected using soda lime (indicating type, 4–8 mesh; Mallinckrodt Baker, Phillipsburg, NJ). Oxygen concentration was monitored daily with an oximeter (Hudson Ventronics

Abbreviations: BAL, bronchoalveolar lavage; **BPD,** bronchopulmonary dysplasia; **FiO_2,** inspired oxygen concentration; **MBC,** minimum bactericidal concentration; **MIC,** minimum inhibitory concentration

5590, Temecula, CA). Pups were evaluated daily for survival and growth for 14 d, then euthanized (see Animal section above), and randomly evaluated for lung weight analysis; *Ureaplasma* culture and PCR of blood, homogenized lung, and bronchoalveolar lavage (BAL); and lung tissue histology, inflammation, and morphometry.

Lung weight analysis. Whole lungs were obtained *via* blunt dissection, weighed immediately, and reweighed after air drying. Final lung dry weight was obtained after consecutive days without weight loss. Lung wet/dry ratio and wet lung/body weight ratio were documented as an index of lung injury (12).

Ureaplasma *cultures.* Hundred microliters of blood was obtained *via* cardiac puncture from each pup.

Lungs were perfused intratracheally with 0.5 mL of a 1:1 mixture of 99% glycerin (Sigma-Aldrich, St. Louis, MO) and normal saline through a 21-gauge needle on a 3-mL syringe and the BAL sample obtained with suction applied after instillation of the mixture (13).

Lung tissue was homogenized between a 60 × 15 mm petri culture dish (Pyrex, Lowell, MA) and a 4-mL glass vial (Kimble Glass Inc., Raleigh, NC) in a sterile fashion (10).

The culture samples (blood, BAL, lung homogenate) were placed in 700 μL of 10B broth and incubated (Precision Scientific Group, Chicago, IL) at 37°C. Samples were examined daily for 2 wk for color change and cultures confirmed on A7 Agar (14).

Ureaplasma *PCR.* All culture (blood, BAL, lung homogenate) samples were prepared for PCR by vortexing briefly before centrifuging at 1000 rpm for 10 s (Beckman Coulter, Fullerton, CA). The supernatant was centrifuged at 1000 rpm for 20 min and pellets were lysed with 20 mM Tris-HCl, 50 mM KCl, 2.5 mM $MgCl_2$, and 0.5% Tween 20. Proteinase K (300 μg/mL; Qiagen, Valencia, CA) was added before 2-h incubation at 60°C, followed by 10 min at 94°C. PCR was performed using primers directed toward the urease genes specific for *Ureaplasma*: primers U4a (antisense primer, 5′-acg acg tcc ata agc aac aac t-3′) and U5s (sense primer, 5′-caa tct gct cgt gaa gta tta c-3′). The reaction mixture was subjected to the following thermal cycling parameters in a PTC-200 model Peltier Thermal Cycler (MJ Research, Waltham, MA): 34 cycles of 30 s at 94°C, 60 s at 60°C, 60 s at 72°C, and then 10 min at 72°C. The PCR product was stored at 4°C and run on 2% agarose gel. Positive and negative DNA controls, and water controls were processed in parallel to detect false negatives and contamination (2). Each assay had a detection limit of approximately five copies per reaction of the respective gene target.

Lung pathology. Lung tissue was formalin fixed, paraffin embedded, and sectioned at 4 μm after intratracheal instillation of 10% zinc formalin at constant pressure (12). Fixative was introduced *via* a transtracheal needle into the lung *in situ*. The trachea tied off to retain the fixative at the filling pressure. Lungs were visualized as they were inflated to ensure no overinflation. The filling pressure used for inflation was a constant 4 cm H_2O. Fixed lungs were stained with hematoxylin and eosin and anti-neutrophil antibody (AbD Serotec, Oxford, United Kingdom) (15). Anti-neutrophil antibody-stained tissue was used to count neutrophils at ×40 in five high-power fields/pup. Hematoxylin and eosin-stained tissue was used to evaluate morphology and inflammation. Lung morphometry was documented by measuring the volume in alveolar space at ×40 in 40 high-power fields/pup using BioQuant Life Science software (BioQuant Image Analysis Corporation, Nashville, TN). All samples were evaluated by a pathologist in a blinded fashion.

Antibiotics. Erythromycin (USP, Rockville, MD) was selected based on historical data and practice (1,6,8) and azithromycin (Baxter, Deerfield, IL) on current and local data (Ref. 16 and Oluola O *et al*., *Ureaplasma* colonization of the placenta and preterm birth, 2007 Pediatric Academic Society Annual Meeting, May 5, 2007, Toronto, Canada, Abstract 615906.3). Antibiotic dose and frequency were based on clinical guidelines (1,6,8,16). Antibiotic powders were dissolved with normal saline per manufacturer recommendation. Fresh stock solutions were prepared daily.

Pharmacokinetics. One-day-old pups received either intraperitoneal erythromycin 20 mg/kg/dose twice a day for 3 d or azithromycin 12 mg/kg/dose daily for 3 d. Blood was collected by cardiac puncture at 1, 5, 7, 23, 25, 29, 31, 47, 49, 53, 55, 72, and 96 h. Blood from two to three pups was pooled per data point, and two data points were obtained for each time point.

Drug level assays. Antibiotic levels were obtained by bioassay (17). Standard dilutions of erythromycin and azithromycin were prepared (17,18). Agar plates were inoculated with *Kocuria rhizophila* (ATCC 9341). Reference and unknown wells were filled, zones of inhibition read, and values obtained from a standard curve (17).

Prevention model. As above, one-day-old pups were placed in 0.21 or 0.8 FiO_2 (Animal Model), 2 h later prophylaxis began with erythromycin, azithromycin, or normal saline (Pharmacokinetics), and 2 h later they received either *Ureaplasma* or 10B and were evaluated (Animal Model).

Statistical analysis. Survival data were analyzed by χ^2 or Fisher exact test and cultures by Kruskal-Wallis. Growth curves were constructed for individual pups (even those who died in the study period), slopes calculated, and means of the slopes for each group compared by one-way ANOVA. One-way ANOVA with Dunnett's multiple comparison tests were performed to analyze lung wet/dry weight and wet lung/body weight ratios. *t* test was used to evaluate anti-neutrophil antibody counts. Results are expressed as mean ± SEM. The sample size estimate of >65 mouse pups per group was based on an untreated survival of 50%, a treated survival of 75%, *p* value of ≤0.05, power of 0.8, and a two-sided comparison. A *p* value ≤0.05 was considered statistically significant for all calculations. MINITAB Release 13.3 (State College, PA) and GraphPad Prism 4 were used for analysis (GraphPad Software, Inc., San Diego, CA).

RESULTS

Model survival. Figure 1 illustrates the survival of suckling mice in the animal model. Pups exposed to 0.8 FiO_2 and infected with *Ureaplasma* had a survival rate of 63% ($n = 70$), whereas pups exposed to 0.8 FiO_2 alone or 0.21 FiO_2 alone had a survival rate of 99% ($n = 71$ and $n = 72$, respectively). Pups exposed to *Ureaplasma* alone had a survival rate of 96% ($n = 67$). The difference between the hyperoxia-exposed and *Ureaplasma*-infected group *versus* each of the other groups was statistically significant ($p < 0.00001$).

Model growth. The 0.8 FiO_2 and *Ureaplasma*-infection group had a weight gain of 0.275 g/d (±0.044, $n = 70$). This rate of weight gain was significantly different from that in the other groups ($p \leq 0.01$), which were 0.385 g/d (±0.007, $n = 67$) for 0.21 FiO_2 and *Ureaplasma*-infection group; 0.391 g/d (±0.008, $n = 71$) for 0.8 FiO_2 and 10B broth group; and 0.380 g/d (±0.009, $n = 72$) for 0.21 FiO_2 and 10B broth group.

Model lung ratios. The lung wet/dry weight ratio in the model pups exposed to 0.8 FiO_2 and *Ureaplasma* infection had a ratio of 8.86 (±3.03, $n = 15$) compared with the 0.21 FiO_2 and *Ureaplasma*-infection group ratio of 4.77 (±0.29, $n = 44$), the 0.8 FiO_2 and 10B broth group ratio of 6.30 (±1.99, $n = 39$), and the 0.21 FiO_2 and 10B broth group ratio of 4.13 (±0.39, $n = 56$). The 0.8 FiO_2 and *Ureaplasma*-infection group had a significantly different lung wet/dry ratio compared with the 0.21 FiO_2 and *Ureaplasma*-infection group ($p \leq 0.05$) and the 0.21 FiO_2 and 10B broth group ($p \leq 0.01$), but not the 0.8 FiO_2 and 10B broth group.

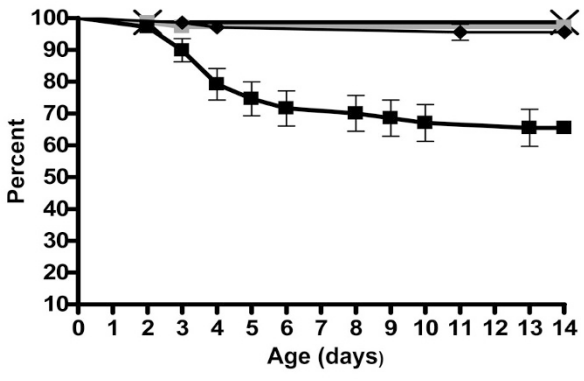

Figure 1. Model survival. Survival of suckling mice over time in days after assignment to model group. *Rectangles*, 0.8 FiO_2 and *Ureaplasma* infection, $n = 70$ (63%); *diamonds*, 0.21 FiO_2 and *Ureaplasma* infection, $n = 67$ (96%); *gray square*, 0.8 FiO_2 and 10B broth, $n = 71$ (99%); and *X*, 0.21 FiO_2 and 10B broth, $n = 72$ (99%). Survival was significantly decreased in the 0.8 FiO_2 and *Ureaplasma*-infected group ($p \leq 0.00001$) *vs* every other group. Data expressed as mean ± SEM.

The wet lung/body weight ratio data of pups exposed to 0.8 FiO_2 and *Ureaplasma* infection had a ratio of 0.018 (±0.001, $n = 15$) compared with the 0.21 FiO_2 and *Ureaplasma*-infection group ratio of 0.14 (±0.001, $n = 44$), the 0.8 FiO_2 and 10B broth group ratio of 0.013 (±0.001, $n = 41$), and the 0.21 FiO_2 and 10B broth group ratio of 0.013 (±0.001, $n = 61$). The difference between the 0.8 FiO_2 exposed and *Ureaplasma*-infected group *versus* each of the other groups was statistically significant ($p \leq 0.01$).

Model cultures. Cultures were obtained in surviving pups at 14 d to evaluate the pervasiveness of *Ureaplasma* in the animal model. Blood cultures and PCR (0.21 FiO_2 and *Ureaplasma*, $n = 25$; 0.8 FiO_2 and *Ureaplasma*, $n = 16$), BAL cultures (0.21 FiO_2 and *Ureaplasma*, $n = 11$; 0.8 FiO_2 and *Ureaplasma*, $n = 14$), and homogenized lung culture (0.21 FiO_2 and *Ureaplasma*, $n = 15$; 0.8 FiO_2 and *Ureaplasma*, $n = 13$) were all negative. The BAL PCR was positive in only 18% (2 of 11) and 29% (4 of 14) of the pups exposed to 0.21 FiO_2 and *Ureaplasma* and 0.8 FiO_2 and *Ureaplasma*, respectively. The homogenized lung PCR was negative in the entire 0.21 FiO_2 and *Ureaplasma* group ($n = 15$) and positive in 15% (2 of 13) of the 0.8 FiO_2 and *Ureaplasma* group. The numbers were small, and there was no statistical difference between *Ureaplasma*-infected groups. Before this study, 10 pups in the 0.8 FiO_2 and *Ureaplasma* group were euthanized and lungs cultured 6 h after the last dose of *Ureaplasma* on d 3, and all had *Ureaplasma* in their lung confirmed by culture and PCR.

Model lung histopathology. Infection with *Ureaplasma* or exposure to oxygen, or both, resulted in significant inflammation compared with nonexposed controls. Infection with *Ureaplasma* and exposure to oxygen appear to demonstrate increased inflammation compared with either one alone (see Figs. 2*A*, *B* and 3*A*, *B*). Lung tissue anti-neutrophil antibody staining found neutrophil counts of 111.4 (±15, $n = 7$) for 0.8 FiO_2 and *Ureaplasma* group, compared with the 0.21 FiO_2 and *Ureaplasma* group 71.3 (±10.6, $n = 7$) and the 0.8 FiO_2 and 10B broth group 67 (±7, $n = 7$). The 0.8 FiO_2 and *Ureaplasma*-infection group had significantly increased lung neutrophil counts compared with the 0.21 FiO_2 and *Ureaplasma*-infection group ($p = 0.049$) and the 0.8 FiO_2 and 10B broth group ($p = 0.025$). There was no statistically significant difference among the groups in lung morphometry (data not shown). Before this study, 10 pups in the 0.8 FiO_2 and *Ureaplasma* group and 10 normal pups not exposed to oxygen or infected were euthanized at 3 d and autopsies performed. The findings in the oxygen exposed and infected pups included the consistent presence of hyaline membranes with occasional fibrin, blood, edema, and large alveoli. The histopathology of the interstitium, perivascular space, and peribronchial space was unremarkable.

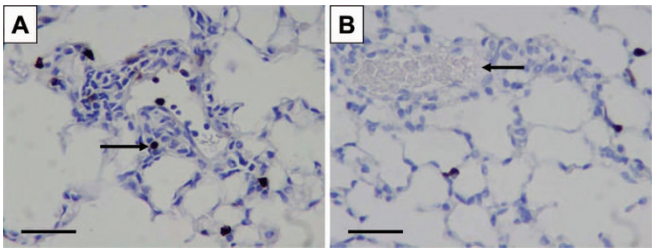

Figure 3. *A*, Histologic appearance of anti-neutrophil antibody-stained lung from 0.8 FiO_2 and *Ureaplasma*-infection group at 14 d of age (×40). *Arrow* points to neutrophils in perivascular space. Bar length in lower left had corner equals 50 μm. *B*, Histologic appearance of anti-neutrophil antibody-stained lung from 0.21 FiO_2 and *Ureaplasma*-infection group at 14 d of age (×40). *Arrow* points to absence of neutrophils in perivascular space. Bar length in lower left had corner equals 50 μm.

Ureaplasma *MIC/MBC.* For this strain of *Ureaplasma*, the MIC and MBC for erythromycin and azithromycin were 62.5 μg/mL and 0.25 μg/mL, respectively.

Pharmacokinetics. Erythromycin serum levels never achieved concentrations necessary for MIC (see Fig. 4). Only azithromycin peak serum levels achieved concentrations necessary for MIC (see Fig. 5).

Prophylaxis survival. The survival rate of pups that received azithromycin prophylaxis (68%) was statistically significant ($p \leq 0.042$) compared with pups that received normal saline (53%; Fig. 6). Survival after erythromycin prophylaxis (56%) was not significantly different from normal saline (53%; $p = 0.87$). Azithromycin prophylaxis (68%) was not statistically different from erythromycin prophylaxis (56%; $p = 0.17$).

Prophylaxis growth. The growth rate of pups was 0.38 g/d (±0.017, $n = 75$) for azithromycin prophylaxis, 0.34 g/d (±0.019, $n = 75$) for erythromycin prophylaxis, and 0.33

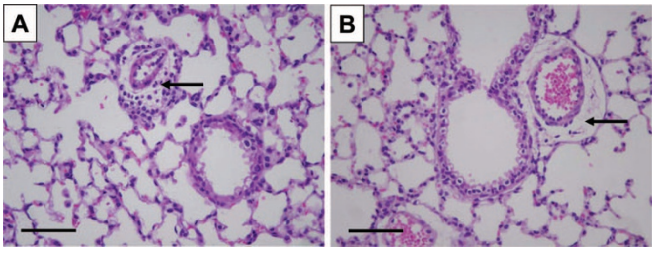

Figure 2. *A*, Histopathologic appearance of hematoxylin and eosin-stained lung from 0.8 FiO_2 and *Ureaplasma*-infection group at 14 d of age (×20). *Arrow* points to neutrophils in perivascular space. Bar length in lower left had corner equals 100 μm. *B*, Histopathologic appearance of hematoxylin and eosin-stained lung from 0.21 FiO_2 and *Ureaplasma*-infection group at 14 d of age (×20). *Arrow* points to absence of neutrophils in perivascular space. Bar length in lower left had corner equals 100 μm.

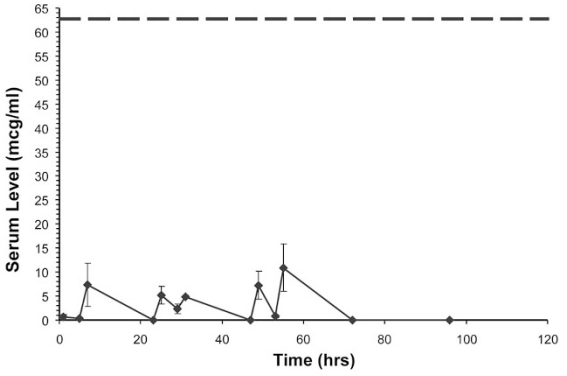

Figure 4. Erythromycin serum concentrations *vs* time in hours in the neonatal mouse. MIC of *Ureaplasma* clinical strain represented by *dashed line* (62.5 μg/mL). Serum levels expressed as mean ± SEM.

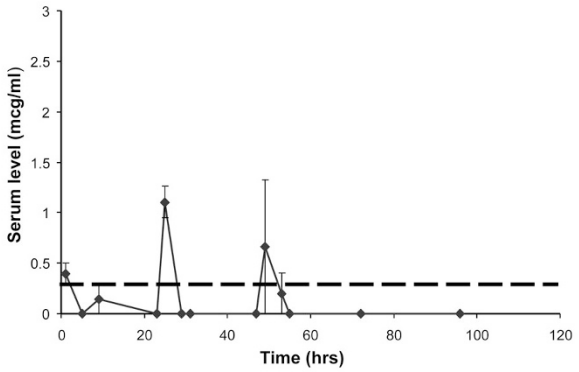

Figure 5. Azithromycin concentrations *vs* time in hours in the suckling mouse. MIC of *Ureaplasma* clinical strain represented by *dashed line* (0.25 μg/mL). Serum levels expressed as mean ± SEM.

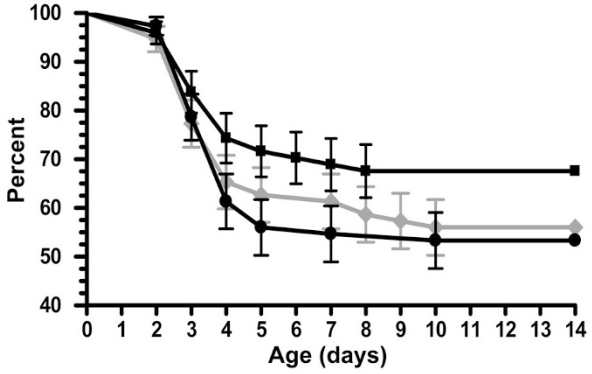

Figure 6. Prophylaxis survival. Survival of suckling mice over time in days after assignment to prophylaxis group. *Circle*, 0.8 FiO_2/normal saline/*Ureaplasma* infection, $n = 75$ (53%); *gray diamond*, 0.8 FiO_2/erythromycin/*Ureaplasma* infection, $n = 75$ (56%); *square*, 0.8 FiO_2/azithromycin/*Ureaplasma* infection, $n = 75$ (68%). Survival was significantly improved in the azithromycin *vs* normal saline ($p \leq 0.042$), but not in the azithromycin *vs* erythromycin ($p = 0.17$) and erythromycin *vs* normal saline ($p = 0.87$) groups. Data expressed as mean ± SEM.

g/d (±0.016, $n = 75$) for normal saline controls. The growth rate of pups who received azithromycin prophylaxis was significantly greater than that of pups who received normal saline ($p \leq 0.05$). There was no statistical difference in the growth rate between azithromycin and erythromycin prophylaxis ($p > 0.05$) or erythromycin and normal saline prophylaxis ($p > 0.05$).

Prophylaxis lung ratios. There were no statistically significant differences in lung ratios between erythromycin and azithromycin prophylaxis groups including lung wet/dry weight ratio or wet lung/body weight ratio (data not shown).

Prophylaxis cultures. In the prophylaxis experiment, the results of blood cultures and blood PCR (erythromycin, $n = 19$; azithromycin, $n = 18$; normal saline, $n = 15$), BAL culture (erythromycin, $n = 12$; azithromycin, $n = 17$; normal saline, $n = 15$), and homogenized lung culture (erythromycin, $n = 5$; azithromycin, $n = 6$; normal saline, $n = 5$) were all negative. BAL PCR was positive in 25% (3 of 12) of erythromycin, and 24% (4 of 17) of azithromycin pups. Homogenized lung PCR were positive in 40% (2 of 5) of erythromycin and 33% (2 of 6) of azithromycin animals. The numbers were small, and there was no significant difference between the groups.

Prophylaxis lung histopathology. There was no statistical difference in lung morphometry between erythromycin and azithromycin prophylaxis groups (data not shown). However, azithromycin prophylaxis resulted in significantly less inflammation based on lower neutrophil counts than normal saline ($p = 0.017$) or erythromycin ($p = 0.039$). Anti-neutrophil antibody staining data for normal saline controls were 92.9 (±7.5, $n = 7$), compared with the erythromycin prophylaxis group count of 90.9 (±9.7, $n = 7$) and the azithromycin prophylaxis group count of 59.3 (±9.4.1, $n = 7$).

DISCUSSION

This study revealed that mouse pups exposed to oxygen and *Ureaplasma* had significantly increased mortality, decreased growth rate, increased lung injury, and increased inflammation compared with pups exposed to *Ureaplasma*, oxygen, or room air alone. This correlates with previous clinical studies that independently link *Ureaplasma* with BPD (19–22) and death (22) despite surfactant treatment (21), and more recent studies and reviews continue to support this link (23–25). These studies showed differences in the studied populations, entrance criteria, and study design. However, the risks of developing chronic lung disease in infants colonized with *Ureaplasma* were similar in all studies.

The possible mechanisms for *Ureaplasma* and hyperoxia interaction that result in increased BPD have not been definitively determined. Hyperoxia may serve as a cofactor for *Ureaplasma* pulmonary dissemination similar to *Pseudomonas* (26) or cause persistence of *Ureaplasma* in the lung (27). In either case, the combination of hyperoxia and *Ureaplasma* appears to potentiate the pulmonary cellular inflammatory response (27) and/or proinflammatory cytokine response (28,29). Azithromycin treatment may limit the dissemination (26) or persistence of *Ureaplasma* in the lung or directly suppress the inflammatory response to hyperoxia (30) or *Ureaplasma*, or both. Our results suggest that azithromycin treatment appears to suppress the overall *Ureaplasma* infection and inflammatory response of *Ureaplasma* and oxygen-induced lung disease. Additional work is needed to determine a precise mechanism.

There are few newborn mouse models of *Ureaplasma*-associated lung disease, and their methods are different from those in this experiment (13,14,27). Previous models used intranasal or intratracheal administration to the organism. We selected intramuscular injection to ensure each animal was infected and not just colonized. The precise mechanism of infection in the human neonate or animal model, *via* airway or systemic circulation, is not known. A recent report observed 17.3% of 23- to 32-wk gestation infants had cord blood culture positive for *Ureaplasma*, suggesting a potential source for systemic infection (31). We measured survival, growth, blood cultures, lung and BAL cultures, lung edema, morphometry, histology, and inflammation, whereas prior studies only evaluated lung culture, histology, inflammation, and BAL culture, histology, and cytokines. Antibiotic prevention or treatment in these mouse models or in any animal model of *Ureaplasma*-associated lung disease has not been evaluated. We selected

prophylaxis because colonization has been reported at or shortly after birth (1). In addition, previous intervention strategies may have failed because treatment was delayed until cultures confirmed infection (8).

Pup growth was significantly decreased with *Ureaplasma* infection and hyperoxia. This growth reduction is similar to what is seen in animal models and neonates with BPD (11,32). We also found that prophylaxis with azithromycin improved growth and survival compared with normal saline controls and erythromycin although the latter was not statistically significant.

Azithromycin may not have been statistically different from erythromycin in prophylaxis survival, growth, and hematoxylin and eosin stain, but lung inflammation was significantly less as measured by anti-neutrophil antibody staining. Differences between the erythromycin and azithromycin groups may have been minimized in part to the macrolide anti-inflammatory properties (30,33) or inadequate dosing of azithromycin. Future investigations should consider increased dosing regimens.

The decrease in lung inflammation with azithromycin compared with erythromycin may reflect the more specific isolation of neutrophils compared with the broad visualization of inflammation using hematoxylin and eosin staining. Although Viscardi *et al.* (13) report on some BAL cytokines and chemokines in their model, further investigation into the prophylaxis of *Ureaplasma* should consider evaluating systemic and airway inflammatory markers in this model.

Blood culture and PCR were all negative. This may be because *Ureaplasma* is an intracellular organism that is mucosally associated. Although cleared from the blood, *Ureaplasma* appears to target the lung and airway and may be delayed in clearance from the lung as demonstrated by persistence of *Ureaplasma* PCR in the lung of surviving pups. Prophylaxis did not alter the persistence of *Ureaplasma* in BAL or lung tissue. This appears similar to the human neonate, where Walsh *et al.* (34) reported that lung biopsy samples from infants were positive even though tracheal aspirates were negative.

Antibiotic pharmacokinetics in mice has not been evaluated. The pharmacokinetics of erythromycin and the MIC of the clinical *Ureaplasma* strain used in this experiment suggest that erythromycin would not be the drug of choice for prophylaxis. *Ureaplasma* strains have shown a variable sensitivity to antibiotics but azithromycin appears to be more effective *in vitro* than erythromycin (16). Azithromycin may be a more appropriate tool in the prophylaxis of *Ureaplasma*, because in a recent study, all clinical strains were sensitive to this antibiotic, whereas 83% were resistant to erythromycin (Oluola O *et al.*, *Ureaplasma* colonization of the placenta and preterm birth, 2007 Pediatric Academic Society Annual Meeting, May 5, 2007, Toronto, Canada, Abstract 615906.3). Our serum antibiotic levels suggest that we may be using a less-than-adequate amount of azithromycin, however, serum levels of azithromycin may not reflect tissue levels. Azithromycin has been observed as having preferential distribution in lung tissue with increased macrophage uptake during infection (18). Future studies of this model should consider determining tissue levels.

The use and safety of azithromycin in the prophylaxis or treatment of *Ureaplasma*-associated lung disease in neonates warrants further investigation. In this prophylaxis model, where the host was infected with an organism that was resistant to erythromycin, azithromycin was an effective prevention strategy.

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