Randomized trial to evaluate azithromycin's effects on serum and upper airway IL-8 levels and recurrent wheezing in infants with respiratory syncytial virus bronchiolitis

Avraham Beigelman, MD, MSCI,[a] Megan Isaacson-Schmid, BSc,[a] Geneline Sajol, BSc,[b] Jack Baty, BA,[c] Oscar M. Rodriguez, MD,[a] Erin Leege, MPH,[a] Kevin Lyons, BSc,[a] Toni L. Schweiger, RN,[b] Jie Zheng, MA,[c] Kenneth B. Schechtman, PhD,[c] Mario Castro, MD, MPH,[b] and Leonard B. Bacharier, MD[a] *St Louis, Mo*

Background: Respiratory syncytial virus (RSV)–induced bronchiolitis in infancy is a major risk factor for recurrent wheezing and asthma. Because azithromycin attenuated neutrophilic airway inflammation in a murine viral bronchiolitis model, demonstration of similar effects in human subjects might provide a strategy for the prevention of postbronchiolitis recurrent wheezing.

Objectives: We sought to investigate whether azithromycin treatment during RSV-induced bronchiolitis reduces serum and nasal lavage IL-8 levels and the occurrence of postbronchiolitis recurrent wheezing.

Method: We performed a randomized, double-masked, placebo-controlled proof-of-concept trial in 40 otherwise healthy infants hospitalized with RSV bronchiolitis who were treated with azithromycin or placebo for 14 days. IL-8 levels were measured in nasal lavage fluid and serum on randomization, day 8, and day 15 (nasal lavage only). The occurrence of wheezing episodes was assessed monthly over the ensuing 50 weeks.

Results: Compared with placebo, azithromycin treatment did not reduce serum IL-8 levels at day 8 ($P = .6$) but resulted in a greater decrease in nasal lavage fluid IL-8 levels by day 15 ($P = .03$). Twenty-two percent of azithromycin-treated participants experienced at least 3 wheezing episodes compared with 50% of participants in the placebo group ($P = .07$). Azithromycin treatment resulted in prolonged time to the third wheezing episode ($P = .048$) and in fewer days with respiratory symptoms over the subsequent year in comparison with placebo (36.7 vs 70.1 days, $P = .01$).

Conclusion: In this proof-of-concept study azithromycin treatment during RSV bronchiolitis reduced upper airway IL-8 levels, prolonged the time to the third wheezing episode, and reduced overall respiratory morbidity over the subsequent year. (J Allergy Clin Immunol 2014;■■■:■■■-■■■.)

Key words: *Azithromycin, IL-8, respiratory syncytial virus, wheezing*

Respiratory syncytial virus (RSV) is the major cause of bronchiolitis in the first year of life.[1-3] Most children infected with RSV experience mild disease that does not require hospitalization; nevertheless, RSV-induced bronchiolitis is the leading cause of hospitalization in infants younger than 1 year of age in the United States.[4,5] Early-life RSV bronchiolitis is a major risk factor for subsequent recurrent wheezing and asthma,[6-10] and this risk is more profound among infants with severe bronchiolitis requiring hospitalization compared with infants with less severe bronchiolitis involving an outpatient encounter.[11,12] Among a high-risk group of infants hospitalized with RSV bronchiolitis, up to 90% of children experience 2 or more wheezing episodes, and almost 50% will be given a diagnosis of asthma by the age of 6 years.[10] Because hospitalized infants have the greatest risk for post-RSV wheezing episodes, they represent an attractive target population in which to explore intervention strategies for the prevention of post-RSV recurrent wheeze.

Multiple therapeutic strategies have been explored with the goal of reducing post-RSV wheezing, most with negative results. Numerous trials of treatments typically used for asthma, such as inhaled corticosteroids (ICSs),[13-15] systemic corticosteroids,[16-18] and montelukast,[19,20] have shown to have limited to no effect on the occurrence of postbronchiolitis wheezing. These medications have minimal effect on noneosinophilic airway inflammation,[21]

From [a]the Division of Allergy, Immunology and Pulmonary Medicine, Department of Pediatrics; [b]the Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine; and [c]the Division of Biostatistics, Washington University School of Medicine and St Louis Children's Hospital.

Supported by Washington University Institute of Clinical and Translational Sciences grant UL1 TR000448 from the National Center for Advancing Translational Sciences (subaward KL2 TR000450) and the Children's Discovery Institute of Washington University and St Louis Children's Hospital. Supported in part (REDCap database) by CTSA grant UL1 TR000448 and Siteman Comprehensive Cancer Center and NCI Cancer Center support grant P30 CA091842.

Disclosure of potential conflict of interest: A. Beigelman is employed by Washington University. M. Castro has received consultancy fees from Asthmatx/Boston Scientific, Genentech, IPS/Holaira, and Neostem; has received or has grants pending from Boston Scientific, Amgen, Ception/Cephalon/Teva, Genentech, MedImmune, Merck, Novartis, GlaxoSmithKline, Sanofi-Aventis, Vectura, Next Bio, and Kalo-Bios; has received payment for delivering lectures from GlaxoSmithKline, Genentech, Boston Scientific, Boehringer Ingelheim, and Teva; receives royalties from Elsevier; and holds stock/stock options from Sparo. L. B. Bacharier has received consultancy fees from Aerocrine, GlaxoSmithKline, Genentech/Novartis, Merck, Schering, Cephalon, DBV Technologies, and Teva; has received or has grants pending from the National Heart, Lung, and Blood Institute/National Institutes of Health AsthmaNet (U10HL098098-01); and has received payment for delivering lectures from Aerocrine, AstraZeneca, Genentech/Novartis, GlaxoSmithKline, Merck, Schering, and Teva. The rest of the authors declare that they have no relevant conflicts of interest.

Clinicaltrials.gov Identifier: NCT01486758.

Received for publication August 12, 2014; revised September 26, 2014; accepted for publication October 2, 2014.

Corresponding author: Avraham Beigelman, MD, MSCI, Division of Allergy, Immunology and Pulmonary Medicine, Department of Pediatrics, Washington University School of Medicine, 660 S Euclid Ave, Campus Box 8116, St Louis, MO 63110. E-mail: beigelman_a@kids.wustl.edu.

0091-6749/$36.00

http://dx.doi.org/10.1016/j.jaci.2014.10.001

Abbreviations used
APW-RSV: Azithromycin to Prevent Wheezing Following RSV Bronchiolitis
BAL: Bronchoalveolar lavage
ICS: Inhaled corticosteroid
IQR: Interquartile range
RSV: Respiratory syncytial virus

which is the dominant pattern seen during bronchiolitis,[22,23] and this might explain the lack of efficacy of these treatments on postbronchiolitis wheezing.

One potential intervention for the prevention of post-RSV wheezing is a macrolide antibiotic, which provides clinical benefits in patients with inflammatory airway diseases, such as cystic fibrosis and diffuse panbronchiolitis, likely through a combination of anti-inflammatory and antimicrobial activities.[24,25] Macrolides also have antineutrophilic activities *in vitro*[24] and within the airway in patients with refractory neutrophilic asthma.[26] Moreover, we have previously reported that in a mouse model of viral bronchiolitis, the macrolide azithromycin decreased neutrophil accumulation in the airway and attenuated neutrophilic inflammation, as evidenced by lower levels of bronchoalveolar lavage (BAL) fluid CXCL1 (previously termed KC), the mouse homologue of the major neutrophil chemoattractant IL-8.[27]

On the basis of these observations, we conducted this proof-of-concept study to evaluate whether these biological effects of azithromycin are evident in infants hospitalized with RSV bronchiolitis. We hypothesized that azithromycin therapy during RSV bronchiolitis would reduce IL-8 levels in serum and upper airway secretions over the following 2 weeks. A secondary goal of this study was to generate a preliminary estimate of the effect size for a potential future trial of azithromycin for the prevention of post-RSV recurrent wheezing.

METHODS

Participants

Potential participants for the Azithromycin to Prevent Wheezing Following RSV Bronchiolitis (APW-RSV) study were initially identified by a positive nasopharyngeal RSV swab result in the St Louis Children's Hospital virology laboratory during 2 consecutive winter RSV seasons (2011-2012 and 2012-2013). This initial screening was performed irrespective of site of care (inpatient, emergency department, or outpatient), clinical indication for obtaining the nasal swab, or the patient's age. On the basis of this initial screen, all infants 1 to 18 months of age with positive nasopharyngeal swab results for RSV and bronchiolitis requiring inpatient care at St Louis Children's Hospital were further screened (Fig 1) by a study coordinator to determine eligibility. Infants were eligible to enroll in the APW-RSV study if they were 1 to 18 months of age, were otherwise healthy, were hospitalized with a first episode of lower respiratory tract symptoms, and had a nasopharyngeal swab (direct fluorescent antibody or multiplex kit) result confirming infection with RSV. Additional eligibility criteria were duration of respiratory symptoms from onset to admission of less than 5 days and randomization within 7 days of the onset of respiratory symptoms. Exclusion criteria included a history of previous wheeze, any previous treatment with corticosteroid (systemic or inhaled), treatment with bronchodilators before the current RSV-induced bronchiolitis episode, use of antigastroesophageal reflux medication, treatment with any antibiotics within the past 2 weeks (4 weeks for macrolide antibiotics), prematurity (gestational age <36 weeks), or any chronic disease (lung, cardiac, renal, or hepatic disease).

During the hospitalization, the infants were treated according to a predefined care path, the St Louis Children's Hospital Bronchiolitis Pathway, a set of orders based on the American Academy of Pediatrics guidelines for treatment of bronchiolitis.[28] The St Louis Children's Hospital Bronchiolitis Pathway is an evidence-based pathway that focuses on supportive treatments, such as nasal suctioning, supplemental oxygen, and intravenous fluids for infants who are not able to feed adequately. A trial of inhaled albuterol is permitted, but the treatment should be discontinued if albuterol does not provide clinical benefit. The pathway strongly discourages use of systemic corticosteroids and antibiotics. All decisions regarding medical treatment during the hospitalization, other than those related to study participation, were made by the child's primary attending physician. The study protocol was approved by the Washington University Institutional Review Board. Participants' parents provided written informed consent, and a data and safety monitoring board monitored the study.

Study design and treatment

The APW-RSV study was a randomized, double-masked, placebo-controlled proof-of-concept trial (Fig 2). All participants, their families, investigators, and study staff were blinded to study medication allocations. Each eligible participant was randomly assigned to receive either placebo or azithromycin in random blocks based on a computer-generated randomization scheme. Study treatments were either azithromycin oral suspension (10 mg/kg once daily for 7 days, followed by 5 mg/kg once daily for additional 7 days; Teva Pharmaceuticals, Sellersville, Pa) or an oral placebo suspension that was matched in taste and appearance to the azithromycin oral suspension. Adherence to study medication was calculated based on measurements of medication bottle weight obtained before and after the treatment phase.

Outcome measures: General overview

The APW-RSV trial included coprimary outcomes, including biological and clinical outcomes. The primary biological outcome of this trial evaluated the effect of azithromycin treatment on serum and nasal lavage fluid IL-8 levels, whereas the effect of treatment on recurrent wheezing was the primary clinical outcome of the study.

IL-8 measurements

Serum and nasal lavage samples for IL-8 measurements were obtained on randomization and 8 days later. An additional nasal lavage sample was obtained on day 15 (Fig 2). The change in serum IL-8 levels from randomization to day 8 was the primary biological outcome, and the changes in nasal lavage fluid IL-8 levels from randomization to day 8 and day 15 were prespecified as secondary outcomes.

Nasal lavage samples were obtained by the study coordinator: each of the child's nostrils was flushed with 3 mL of 0.9% saline, and then the nasal fluid and upper airway secretions were aspirated with a syringe bulb.

Serum and nasal lavage fluid IL-8 levels were measured with the BD human cytometric bead array (BD Biosciences, San Diego, Calif), according to the manufacturer's instructions.[29,30] Serum IL-8 levels were measured with the Enhanced Sensitivity IL-8 Flex Set (lower detection limit, 69.9 fg/mL), whereas nasal lavage fluid IL-8 levels were measured by using the IL-8 Flex Set (lower detection limit of 1.2 pg/mL). Data acquisition occurred on an LSR II BD flow cytometer with DIVA software. Results were analyzed with FCAP Array Software (version 3.0, BD Biosciences).

Clinical outcomes assessment

The primary clinical outcome was the proportion of participants who experienced 2 or more additional wheezing episodes, assessed by monthly telephone calls over the 50 weeks after the treatment period and at a final clinic visit 1 year after randomization (Fig 2). The proportion of children who experienced 3 or more wheezing episodes was specified as a secondary outcome. We defined the occurrence of a wheezing episode each time