Published in final edited form as:

J Allergy Clin Immunol. 2015 October ; 136(4): 1129–1131. doi:10.1016/j.jaci.2015.06.011.

Does Azithromycin Modify Viral Load During Severe RSV Bronchiolitis?

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Keywords

Azithromycin; respiratory syncytial virus (RSV); wheezing; asthma; viral load

To the Editor

Early life respiratory syncytial virus (RSV) bronchiolitis is a major risk factor for subsequent recurrent wheezing and asthma^{1, 2}. The highest asthma risk occurs in infants with severe bronchiolitis requiring hospitalization³: up to 75% of hospitalized infants experience at least 3 additional wheezing episodes and almost 50% are diagnosed with asthma by the age of 7 yrs¹.

We recently reported the results of a proof-of-concept trial, “Azithromycin for the Prevention of Recurrent Wheezing following RSV Bronchiolitis (APW-RSV)”, in which azithromycin (AZM) treatment of infants hospitalized with RSV bronchiolitis resulted in a lower likelihood of subsequent recurrent wheezing⁴. Anti-inflammatory effects of AZM were detected in that study: relative to placebo, AZM treatment resulted in a greater decline in nasal lavage IL-8 levels by day 15 ($p=0.03$)⁴. However, other mechanisms through which AZM may exert this protective effect remain uncertain. Previous laboratory investigations have suggested that macrolides have *in-vitro* anti-viral activity, as pre-treatment of *ex vivo* human respiratory epithelial cells with AZM⁵ or clarithromycin⁶ reduced replication and

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release of RSV⁶ and rhinovirus⁵. In addition, AZM pre-treatment reduces rhinovirus replication in cystic fibrosis bronchial epithelial cells⁷. However, potential *in vivo* anti-viral effects of macrolides have not been investigated in humans infected with respiratory viruses. Therefore, we hypothesized that our finding of AZM's reduction in post-RSV recurrent wheeze⁴ was mediated, at least in part, by anti-viral activity.

In this current study, we aimed to address two research questions. Does AZM have antiviral activity as demonstrated by lower RSV loads in serial nasal lavage samples obtained during RSV bronchiolitis? Does this potential anti-viral activity mediate the reduction in recurrent wheeze detected in our proof-of-concept trial?

A detailed description of the APW-RSV study has been reported elsewhere⁴. Briefly, this was a randomized, double-masked, placebo-controlled, proof-of-concept trial involving 40 infants with RSV bronchiolitis. Eligible infants were 1-18 months of age, otherwise healthy, and hospitalized for the first episode of bronchiolitis with confirmed RSV infection. Infants with history of prematurity were excluded. Study treatments were either AZM oral suspension 10 mg/kg once daily for 7 days, followed by 5 mg/kg once daily for 7 additional days, or an oral placebo suspension. AZM therapy was well tolerated, as mild gastrointestinal adverse events (diarrhea, vomiting, or abdominal pain) during the active treatment phase were recorded in 15 children (7 azithromycin, 8 placebo); none warranted discontinuation of study medication⁴. 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.

RSV load was measured in nasal lavage samples obtained on randomization, day 8, and day 15, using the RealStar RSV A/B RT-PCR Kit, Altona Diagnostics (Hamburg, Germany). The occurrence of wheezing episodes was assessed as we have previously done¹. The log-transformed RSV loads at three time points were compared between the two treatment groups using mixed-model repeated-measures analysis of covariance with the baseline viral load and duration of respiratory symptoms prior to randomization as covariates.

Complete sets of nasal wash samples obtained before (at randomization), during (day 8), and after (day 15) the study treatments were available for 39 participants (19 AZM, 20 placebo). Study participants were 3.8 ± 2.9 months of age, and 59% were males. Other characteristics of study participants are described in Table 1. No significant differences were noted in baseline characteristics between the treatment groups. Nasal RSV loads during the study period are presented in Figure 1. Mean RSV loads at randomization did not differ between the treatment groups ($p=0.80$). Mean viral loads declined in both groups from randomization to day 8, and further to day 15. Compared to placebo, the AZM group had a higher RSV load on day 8 ($p<0.01$), whereas RSV loads were comparable between groups on day 15 ($p=0.33$).

In this clinical scenario, AZM therapy did not exert anti-viral effects in terms of accelerating the reduction in RSV load among infants with severe bronchiolitis. AZM therapy was associated with less RSV clearance from the airway, albeit in a transient and time-dependent manner, as higher RSV loads were measured in the AZM group on day 8, while comparable

RSV loads were measured in the AZM and placebo groups on day 15. The reason for this unexpected finding is unknown, but we speculate that it might be related to the presumptive anti-inflammatory effects of AZM, which could result in reduced levels of anti-viral mediators. Nevertheless, the effect of the intervention was very temporary, as comparable RSV loads were measured in the two groups on day 15. To our knowledge, this is the first study investigating the potential *in-vivo* anti-viral activity of macrolide in humans infected with respiratory viruses. The differences between our findings and previous reports describing *ex-vivo* anti-viral activity of macrolides⁵⁻⁷ could be related to difference in timing of interventions, as the *ex-vivo* studies used pre-treatment of the epithelial cell before the infection, while our clinical trial involved treatment during ongoing RSV infection⁴. Therefore, a follow up study may incorporate an earlier initiation of macrolide therapy.

Our measured endpoint for the antiviral effect was the level of RSV RNA as measured by RT-PCR. While quantitative RT-PCR has been found to correlate with the level of infectious virus⁸, it is possible but not likely that direct measurement of viable virus by quantitative culture might have shown an effect that was missed by the measurement of viral RNA.

Previous reports correlated higher RSV load in respiratory secretions, measured during the first 3 days of hospitalization⁹ or within 2-4 days from onset of symptoms¹⁰, with more severe courses of acute bronchiolitis. We found no significant difference in the duration of hospitalization between treatment groups (mean (SD) 58.0 (41.4) hrs in AZM group vs. 68.5 (63.2) hrs in placebo group, $p=0.54$). Our results do not contradict these previous reports since at randomization, at a mean of 5.5 days into the illness (Table 1), viral load did not differ between the treatment groups. The transient increase in RSV load in the AZM group was noted only in the recovery phase (day 8) and likely did not affect acute bronchiolitis course. However, as we did not perform serial daily measurements of RSV loads, it remains unknown whether peak RSV load among our study participants was associated with acute bronchiolitis severity.

Viral load measurements were performed on upper airway samples since technical and ethical limitations precluded obtaining lower respiratory tract samples in young infants. We recognize upper airway samples may not directly represent the viral load in the lower airway. Nevertheless, we believe that if azithromycin had an antiviral effect, it would be evident in either the upper or lower airway. In addition, previous reports^{9, 10} support the premise that RSV load measurements in the upper airway are informative as these were related to acute disease severity, and thus may serve as a valuable surrogate of lower airway viral load.

A major strength of our study is the use of a homogenous study population, as we enrolled only otherwise healthy full-term infants experiencing acute RSV bronchiolitis and the prospective collection of biological samples and clinical outcomes in a well-characterized cohort. The relatively small sample size is a limitation; nevertheless, the detection of significant difference in RSV load on day 8 suggests adequate power to detect differences in viral loads between the treatment groups. However, a definitive exclusion of anti-viral properties of macrolides will require confirmation in a larger cohort. In average, the AZM

treatment was initiated at day 6 of illness; therefore, we cannot completely exclude that earlier initiation of therapy would demonstrate an antiviral effect. While the homogeneity of our study population is strength of this study, it is also possible that an antiviral effect might be evident in different populations, including other age or ethnic groups. In addition, it is possible that an antiviral effect might be more evident in certain RSV subtypes that might not have been represented in our study population. Finally, while AZM does not appear to exert anti-viral effects in this population, the mechanism(s) by which macrolides provide anti-inflammatory effects remains uncertain. Future studies including a non-macrolide antibiotic may be helpful in elucidating whether these effects are independent of antimicrobial actions.

In summary, AZM therapy in infants with severe bronchiolitis did not facilitate RSV clearance from the upper airway. As such, we conclude that the beneficial effect of AZM on the reduction in the occurrence of post-RSV recurrent wheeze noted in our proof-of-concept study⁴ was not mediated by direct anti-viral activity of AZM, and may have been mediated by other mechanisms, such as anti-inflammatory effects and airway microbiome modification.

Acknowledgments

Source of funding: Supported by the Washington University Institute of Clinical and Translational Sciences grant UL1 TR000448 from the National Center for Advancing Translational Sciences- sub award KL2 TR000450, and the Children's Discovery Institute of Washington University and St. Louis Children's Hospital. Supported in part (REDCap data base) by the CTSA Grant UL1 TR000448 and Siteman Comprehensive Cancer Center and NCI Cancer Center Support Grant P30 CA091842.

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Abbreviations

AZM	azithromycin
RSV	Respiratory syncytial virus

Figure 1. Mean log₁₀ RSV nasal lavage loads on randomization, day 8, and day 15 among the APW-RSV trial participants. * p<0.01.

Table 1
Baseline characteristics^a of the APW-RSV study participants

	All participants	Azithromycin (n=19)	Placebo (n=20)
Age at enrollment (months) ^b	3.8 (2.9)	3.7 (3.7)	3.9 (2.0)
Gender (male)	59.0%	47.4%	70.0%
Race (Caucasian)	64.1%	63.2%	65.0%
Birth weight (kg) ^b	3.4 (0.5)	3.3 (0.6)	3.4 (0.4)
Length of pregnancy (wks) ^a	38.8 (1.4)	38.9 (1.3)	38.7 (1.4)
Maternal smoking during pregnancy	7.7%	10.5%	5.0%
History of breast feeding	28.2%	31.6%	25.0%
Tobacco smoke exposure	35.9%	42.1%	30%
Pet exposure	61.5%	63.1%	60.0%
History of eczema	17.9%	21.1%	15.0%
Parental history of asthma	41.0%	42.1%	40.0%
Duration of respiratory symptoms prior to randomization (days) ^b	5.4 (1.4)	5.2 (1.3)	5.5 (1.4)
Duration of hospitalization (hours) ^b	63.4 (53.2)	58.0 (41.4)	68.5 (63.2)
Lowest O2 saturation on room air ^b	90.8 (4.3)	91.4 (4.9)	90.3 (3.6)
<i>RSV type:</i>			
RSV A	77%	70%	84%
RSV B	23%	30%	16%

Data are expressed as proportion of children in each group except as noted.

^a p>0.05 for the baseline characteristics comparisons between the treatment groups.

^b Data represents the mean (SD).