

Letter to the Editor

Does azithromycin modify viral load during severe respiratory syncytial virus bronchiolitis?

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 years.¹

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 wheezing.⁴ 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 ($P = .03$).⁴ However, other mechanisms through which AZM may exert this protective effect remain uncertain. Previous laboratory investigations have suggested that macrolides have *in vitro* antiviral activity because pretreatment of *ex vivo* human respiratory epithelial cells with AZM⁵ or clarithromycin⁶ reduced replication and release of RSV⁶ and rhinovirus.⁵ In addition, AZM pretreatment reduces rhinovirus replication in cystic fibrosis bronchial epithelial cells.⁷ However, potential *in vivo* antiviral 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 antiviral activity.

In this present study, we aimed to address 2 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 antiviral 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 elsewhere.⁴ Briefly, this was a randomized, double-masked, placebo-controlled, proof-of-concept trial involving 40 infants with RSV bronchiolitis. Eligible infants were aged 1 to 18 months, otherwise healthy, and hospitalized for the first episode of bronchiolitis with confirmed RSV infection. Infants with a 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 medication.⁴ 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.

The 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 monthly as we have previously done.¹ The log-transformed RSV loads at 3 time

points were compared between the 2 treatment groups using mixed-model repeated-measures analysis of covariance with the baseline viral load and the duration of respiratory symptoms before 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 aged 3.8 ± 2.9 months, 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 Fig 1. Mean RSV loads at randomization did not differ between the treatment groups ($P = .80$). Mean viral loads declined in both groups from randomization to day 8, and further to day 15. Compared with placebo, the AZM group had a higher RSV load on day 8 ($P < .01$), whereas RSV loads were comparable between groups on day 15 ($P = .33$).

In this clinical scenario, AZM therapy did not exert antiviral 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, because higher RSV loads were measured in the AZM group on day 8 whereas 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 antiviral mediators. Nevertheless, the effect of the intervention was very temporary because comparable RSV loads were measured in the 2 groups on day 15. To our knowledge, this is the first study investigating the potential *in vivo* antiviral activity of macrolides in humans infected with respiratory viruses. The differences between our findings and previous reports describing *ex vivo* antiviral activity of macrolides⁵⁻⁷ could be related to difference in the timing of interventions, because the *ex vivo* studies used pretreatment of the epithelial cell before the infection whereas our clinical trial involved treatment during ongoing RSV infection.⁴ Therefore, a follow-up study may incorporate an earlier initiation of macrolide therapy.

Our measured end point for the antiviral effect was the level of RSV RNA as measured by RT-PCR. Although quantitative RT-PCR has been found to correlate with the level of the infectious virus,⁸ it is possible but not likely that direct measurement of the 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 to 4 days from the onset of symptoms,¹⁰ with more severe courses of acute bronchiolitis. We found no significant difference in the duration of hospitalization between treatment groups (mean $\pm$ SD, 58.0 ± 41.4 hours in the AZM group vs 68.5 ± 63.2 hours in the placebo group; $P = .54$). Our results do not contradict these previous reports because 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 the RSV load in the AZM group was noted only in the recovery phase (day 8) and likely did not affect the acute bronchiolitis course.

TABLE I. Baseline characteristics* of the APW-RSV study participants

Characteristic	All participants	Azithromycin (n = 19)	Placebo (n = 20)
Age at enrollment (mo) [†]	3.8 ± 2.9	3.7 ± 3.7	3.9 ± 2.0
Sex: male	59.0%	47.4%	70.0%
Race: Caucasian	64.1%	63.2%	65.0%
Birth weight (kg) [†]	3.4 ± 0.5	3.3 ± 0.6	3.4 ± 0.4
Length of pregnancy (wk)*	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 before randomization (d) [†]	5.4 ± 1.4	5.2 ± 1.3	5.5 ± 1.4
Duration of hospitalization (h) [†]	63.4 ± 53.2	58.0 ± 41.4	68.5 ± 63.2
Lowest O ₂ saturation on room air [†]	90.8 ± 4.3	91.4 ± 4.9	90.3 ± 3.6
RSV type			
RSV A	77%	70%	84%
RSV B	23%	30%	16%

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

* $P > .05$ for the baseline characteristics comparisons between the treatment groups.

[†]Data represent mean ± SD.

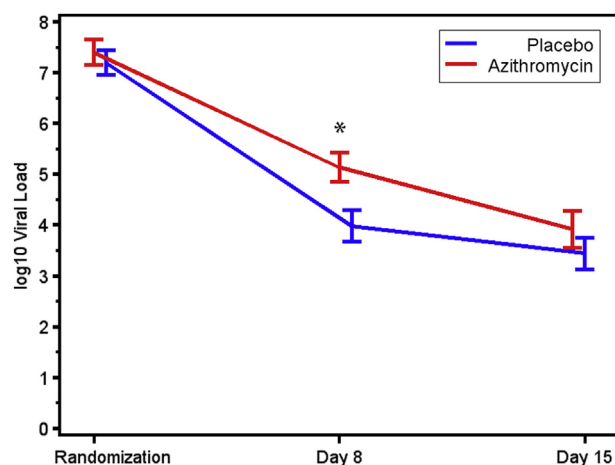


FIG 1. Mean log₁₀ RSV nasal lavage loads on randomization, day 8, and day 15 among the APW-RSV trial participants. * $P < .01$.

However, because we did not perform serial daily measurements of RSV loads, it remains unknown whether the peak RSV load among our study participants was associated with acute bronchiolitis severity.

Viral load measurements were performed on upper airway samples because technical and ethical limitations precluded obtaining lower respiratory tract samples in young infants. We recognize that 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 airway or the lower airway. In addition, previous reports^{9,10} support the premise that RSV load measurements in the upper airway are informative because 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 homogeneous study population because we enrolled only otherwise healthy full-term infants experiencing acute RSV bronchiolitis. In addition, biological samples and clinical outcomes were prospectively

collected in a well-characterized cohort. The relatively small sample size is a limitation; nevertheless, the detection of a significant difference in the RSV load on day 8 suggests adequate power to detect differences in viral loads between the treatment groups. However, a definitive exclusion of antiviral 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. Although the homogeneity of our study population is a 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, although AZM does not appear to exert antiviral effects in this population, the mechanism(s) by which macrolides provide anti-inflammatory effects remains uncertain. Future studies including a nonmacrolide antibiotic may be helpful in elucidating whether these effects are independent of antimicrobial actions.

In summary, AZM therapy in infants with severe RSV 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 antiviral activity of AZM, and may have been mediated by other mechanisms, such as anti-inflammatory effects and airway microbiome modification.

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