Azithromycin, *Ureaplasma* and chronic lung disease of prematurity: a case study for neonatal drug development

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**ABSTRACT**

Chronic lung disease of prematurity (CLD) remains a major cause of morbidity and mortality in preterm infants. *Ureaplasma* has received intermittent attention over the last two decades as a possible contributory factor. In addition, pulmonary inflammation is associated with the development of CLD. The macrolide azithromycin provides an attractive option to determine if it can decrease the development of CLD as it has both anti-inflammatory and anti-infective properties. In this article, the authors review the evidence for the role of *Ureaplasma* in the development of CLD and the obstacles faced in the development of a drug before it reaches clinical practice.

**INTRODUCTION**

Advances in neonatal care have improved the survival of extremely preterm infants, but morbidity1 including neurodevelopmental sequelae and chronic lung disease of prematurity (CLD), also known as bronchopulmonary dysplasia, remains significant. CLD is associated with prolonged hospital stay and often a need for domiciliary oxygen,2 and places a significant burden on the family. Indeed, CLD was identified as having the largest therapeutic gap in a recent survey of UK neonatologists.3 The Paediatric Regulation from the European Commission has stimulated work on systematic drug development for neonates. It is now feasible to obtain a marketing authorisation for off-patent medicines for use in children.4,5 The aims of this review are:

1. to outline the state-of-the-art for one potential intervention for CLD and
2. to use this intervention as a case study for the current approach to drug development for neonates in the context of developing a paediatric investigation plan with a view to applying for a marketing authorisation.

Multiple factors appear to contribute to the development of CLD. In infants who are at risk of developing CLD, one frequent finding is colonisation of the preterm lung with the microbe *Ureaplasma*.6,7 *Ureaplasma* belongs to the class of Mollicutes which are thought to be the smallest free-living, self-replicating organisms. As they do not have a cell wall, they are limited to a parasitic existence in eukaryotic cells. *Ureaplasma urealyticum*, the only species known to infect humans, was recently subdivided into two separate species, *U urealyticum* and *U parvum*, on the basis of their 16S ribosomal RNA gene sequences.8

Two meta-analyses have suggested an association between the presence of pulmonary *Ureaplasma* and the development of CLD.9 10 The more recent report by Schelonka et al which included 23 studies and 2216 babies,10 showed a relative risk of 2.83 (95% CI 2.29 to 3.51) for CLD diagnosed as oxygen dependency at 28 days of life. For the 751 infants who were oxygen-dependent at 36 weeks postconceptional age, the relative risk was 1.62 (1.13 to 2.33). The studies, however, are disparate with most calling for a definitive randomised trial to assess if eradication of pulmonary *Ureaplasma* reduces the rates of CLD.

In addition, lung inflammation during the first week of life is the hallmark of preterm babies who subsequently develop CLD.11 12 Tafari and colleagues described the isolation of *Ureaplasma* from the lungs of stillborn infants with pneumonitis.13 Further support for the contributory role of *Ureaplasma* in human disease comes from demonstration of a specific IgM response in the neonate resulting in radiographic changes indicative of pneumonia in culture positive infants and demonstration of the organism in lung tissue by immunofluorescence and electron microscopy.14 15 Animal models have contributed substantially to understanding of the inflammatory processes that are triggered by *Ureaplasma* in the mammalian respiratory tract, primarily in mouse, sheep and primate models.16 17 For example, Novy et al used a rhesus monkey model of antenatal *Ureaplasma* infection in the amniotic fluid.17 They demonstrated histopathological findings of chorioamnionitis, a systemic fetal inflammatory response and pneumonitis, which worsened as the duration of in utero infection increased.

Initial clinical trials in preterm neonates used erythromycin, a macrolide antibiotic commonly used to treat *Ureaplasma*. Two randomised controlled trials were included in the Cochrane review by Mabanta et al which examined studies that treated *Ureaplasma* to decrease the rate of CLD.18 In the first study, 75 ventilated infants of less than 30 weeks gestation whose *Ureaplasma* status was unknown when treatment commenced, were randomly assigned to receive erythromycin intravenously for 7 days or to no treatment.19 Nine infants (15%) were positive for *Ureaplasma*. Both groups had similar rates of CLD and erythromycin did not decrease CLD severity.19 In the second study, erythromycin was commenced once *Ureaplasma* status was known. Endotracheal aspirates and nasopharynx swabs from ventilated preterm infants (n=155; median gestational age
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(8GA) 26 (range 23–29 weeks) were cultured for Ureaplasma.20 Colonised infants (29/155, 19%) were randomly assigned to treatment with intravenous or oral erythromycin 40 mg/kg/day. Erythromycin treatment was effective in eradicating colonisation in 12/14 (86%) of the treated infants. Treatment with erythromycin reduced colonisation but did not significantly alter the length of time with supplemental oxygen.

Neither trial showed a statistically significant effect of erythromycin on CLD, death or the combined outcome CLD or death. Since the two studies differed markedly in their design, the results were not combined in the meta-analyses. Neither study noted adverse effects for 7–10-day courses of erythromycin. While neither study supports a role for erythromycin in CLD, they provide evidence of tolerability. These studies are at best seen as early phase clinical trials rather than as underpowered tests of efficacy. Other approaches to this problem have been suggested.

RATIONALE FOR USE OF AZITHROMYCIN IN NEONATES

Another potential drug, azithromycin, has anti-infective action against microbes including Ureaplasma in older age groups and also has potent anti-inflammatory actions utilised in many conditions including respiratory diseases such as cystic fibrosis.21-23 Azithromycin is actively concentrated within alveolar macrophages and thus is attractive to treat pulmonary conditions.24 If Ureaplasma colonisation is on the causal pathway to the development of CLD, then azithromycin may reduce the incidence of CLD in combination with its anti-inflammatory effects.

Proof-of-mechanism for azithromycin as a treatment for Ureaplasma in the newborn is suggested by work such as that of Sadowsky who injected U parvum into the amniotic fluid of a rhesus monkey.25 Azithromycin was administered to mothers at a dose of 12.5 mg/kg/12 hours for 10 days. It eradicated the Ureaplasma from the amniotic fluid with significantly decreased pneumonitis.25 However, macrolides in pregnant women have not shown an alteration in maternal outcomes.26 27 Antenatal exposure to erythromycin close to birth improved some neonatal outcomes in the ORACLE trial.28 29 Antenatal exposure to azithromycin in the second trimester was not associated with any differences in outcomes in the APPLLE trial.30 Postnatal azithromycin needs further evaluation in an adequately powered clinical trial.

Since pulmonary inflammation is thought to play an important role in the development of CLD and azithromycin is thought to modify inflammation, Ballard et al performed a pilot double-blind, randomised, placebo controlled trial which specifically excluded neonates colonised by Ureaplasma.31 Infants (birth weight ≤1000 g) were randomised to treatment or placebo within 12 h of beginning intermittent mandatory ventilation and within 72 h of birth. The treatment group received azithromycin 10 mg/kg/day for 7 days followed by 5 mg/kg/day until the infant no longer required mechanical ventilation or supplemental oxygen, to a maximum of 6 weeks. Mortality, incidence of CLD and other morbidities were not significantly different between groups. Postnatal steroid use was significantly less in the treatment group (31%, 6/19) versus the placebo group (62%, 10/16) (p=0.05) as was the duration of mechanical ventilation (median 13 days (range 1–47 days) vs 35 days (range 1–112 days)) (p=0.02). Importantly, the study did not report any significant adverse effects of the use of azithromycin for up to 6 weeks. In a subsequent study, Ballard and colleagues randomised 211 neonates with birth weight <1250 g to azithromycin or placebo irrespective of colonisation status32 and reported an improvement in CLD rates in Ureaplasma colonised infants with wide confidence intervals. Ballard’s work indicates that azithromycin may affect surrogate outcomes, provides proof-of-concept for the anti-inflammatory effects of azithromycin in preterm neonates and demonstrates the tolerability of azithromycin in the target population.33 This extends the proof-of-concept to a group comprising Ureaplasma colonised and non-colonised neonates.

Clearly, at least one sufficiently powered randomised controlled trial is needed to address the role of azithromycin in preterm neonates and would have support from the neonatal community.33 However, before a large randomised controlled trial can be conducted, a number of decisions need to be taken so that a large trial has the best chance of giving a clear answer.

WHICH FORMULATION SHOULD BE USED?

The development of an age-appropriate formulation is central to the paediatric investigation plans introduced under the EU regulation. The plans are guided by a reflection paper: Formulations of Choice for the Paediatric Population.34 To ensure an appropriate formulation, it is necessary to consider excipients, the optimal concentration of the formulation, the required characteristics of infusions pumps and procedures according to the concentration of the formulation and the local tolerance and toxicity of the presentation.

WHAT IS THE ROLE OF ANIMAL WORK IN THE DEVELOPMENT PLAN?

Animal work, often labelled as preclinical work, needs to be considered for drug licensing in the EU.35 Preclinical testing of drugs for paediatric indications is likely to focus on development toxicity and short- and long-term safety. This is different from studies designed to elucidate the mechanism and test proof-of-concept.34 35 The tests performed often include physiological tests, psychomotor development, learning and memory, and a dose ranging pilot study. However, these may not be required for azithromycin as it is already on the market.

WHICH DOSAGE REGIMEN?

A wrong dose will not work! Before use in any trials, it is essential to establish an appropriate dose. Pharmacokinetics (PK; ie, how the body handles the drug) specifically for neonates are essential.36 The value of examining drug disposition is illustrated by a recent report of PK in neonates of metronidazole which found that differences in clearance according to GA indicated that different optimal dosage regimens are required at different GAs.37

With respect to azithromycin, there are two steps to consider: delivery and efficacy. Delivery will depend on a similar time-concentration curve as in older age groups. This is summarised by the area under the time-concentration curve (AUC). For children aged 0.5–16 years, the AUC did not differ with age for a single dose of 10 mg/kg.38 In contrast, the pilot PK data which are available in extremely preterm neonates, suggest that the AUC is markedly greater than in older age groups. This reflects the less extensive clearance of azithromycin and also a smaller volume of distribution.39 The net effect of these effects can only be determined empirically.

In refining the PK model before a large trial, a balance must be struck between safety and benefit. Under the European regulation, the European Medicines Agency (EMEA) has set

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up a Paediatric Committee to balance the scientific and patient welfare issues in paediatric drug development.

**SHOULD WE TREAT INFECTION OR INFLAMMATION?**

Azithromycin has both anti-infective and anti-inflammatory effects. If a trial is chosen to effect both actions, then duration of treatment needs to be optimised to cover both. The data available to inform this decision are:

1. Pulmonary inflammation is present early in life and does not settle within 3–7 days of age.30 41
2. Most previous studies using macrolides to eradicate *Ureaplasma* have used 7–14 days of treatment. Ballard’s trials continued azithromycin therapy for up to 6 weeks.31 32
3. Hassan et al found that a single dose of 10 mg/kg of azithromycin did not affect inflammatory markers.49
4. Prolonged antibiotic exposure is likely to have adverse consequences.

Many clinicians would find it difficult to justify continuing antibiotics for the length of time used by Ballard et al because of the risk of selecting for resistant strains and of potential adverse effects. Caution is advised by the retrospective cohort study of 5693 neonates born at less than 1000 g42 which showed that increasing duration of empiric antibiotics, started within 3 days of birth among babies with negative blood cultures, was associated with increasing risk of necrotising enterocolitis and death.

**WHAT INCLUSION CRITERIA SHOULD WE USE?**

It is important to include preterm infants at high risk of developing CLD, particularly those who are receiving respiratory support at birth. It is difficult to recruit immediately after birth. Inclusion of infants at highest risk of developing CLD, that is, with a GA of 28 weeks or less, receiving respiratory support within 12 h of birth and recruited within 48 h of birth (with first dose administered within 72 h of birth) would seem reasonable. Those infants who may develop respiratory disease due to underlying conditions such as congenital, surgical or cardiac anomalies may be reasonably excluded.

**WHICH MICROBIOLOGICAL ASSESSMENTS SHOULD BE DONE?**

It would be crucial to account for pulmonary colonisation by *Ureaplasma* among the secondary, explanatory outcomes. A nasogastric tube is almost always inserted within the first few hours of age in such babies and thus provides an ideal sample to reflect the antenatal environment, with tracheal or nasopharyngeal aspirates for reflection of the postnatal environment.43 44 This should permit secondary analysis of the effect of colonisation status on outcome and identify the prevalence of *Ureaplasma* antibiotic resistance.45 46 Follow-up sampling to test for treatment cure is essential but may be limited by sample availability. Standard practice for test-of-cure is to examine whether the antimicrobial has been effective three half-lives after treatment stops.

**WHICH END POINTS?**

Any chosen end points should have clinical relevance, should be reproducible and include both respiratory and non-respiratory secondary end points. One option is to use a combined outcome (death or CLD), another option is to use a single outcome (CLD) and use survival analysis to adjust for death. Death may be unrelated to CLD, so that a combined outcome may underestimate the difference that is due to the trial medication. On the other hand, death may be related to CLD, in which case a composite outcome would be a conservative approach. Secondary end points should include length of oxygen dependency, hospital stay and respiratory support with intubated or non-invasive mechanical ventilation (including continuous positive airway pressure), drug usage including postnatal corticosteroids and antibiotics, infection rates, intraventricular haemorrhage, necrotising enterocolitis and retinopathy of prematurity. For the diagnosis of CLD, it is important to use a standardised physiological end point, as recommended by the National Institutes of Health consensus47 or a physiological test.48 49

It is important to follow-up after discharge. CLD at 36 weeks is an important outcome that reflects hospital costs and predicts subsequent events, so it is a reasonable outcome to use to assess efficacy. Outcomes to 2 years, for example, are important to assess safety and provide preliminary evidence of effectiveness.

**WHAT SHOULD THE SAMPLE SIZE BE FOR LARGE CLINICAL TRIALS?**

Issues to consider include rates of CLD/death. It may be advantageous to take account of the proportion of participants who have *Ureaplasma* colonisation but may not be available within the treatment window. The effect of colonisation may best be considered as a prespecified secondary analysis. Uncertainties in sample size often remain. As an estimate, between 650 and 700 preterm infants of 28 weeks or less gestation are likely to be required in an adequately powered randomised controlled trial, which clearly may need modification when colonisation by pulmonary *Ureaplasma* is factored into the calculations. Prior to protocol finalisation, sample size and power can be modelled in light of the PK study, a feasibility survey among participating centres and the available literature. A range of analyses is required. The fundamental interpretation of trials for superiority should be based on an intention-to-treat analysis. This evaluation will need to be supplemented by per protocol analyses and analyses of microbiologically evaluable participants.

**HOW SHOULD SAFETY BE ASSESSED?**

Safety assessments are central to drug development. The choice of safety assessments and their timing should be based on risk assessment which includes the known safety issues with azithromycin and the characteristics of the target population (comorbidity is high among babies born at less than 29 weeks gestation and should not be ascribed to azithromycin unless there is clear evidence of this).

**SHOULD WE AIM FOR A MARKETING AUTHORISATION?**

A marketing authorisation provides assurance that pharmaceutical quality, safety and efficacy have been assessed by independent experts, and dosage and interactions considered. Conventional peer review is not sufficient to meet these needs.50 Marketing authorisation holders have a range of duties to monitor the usage of each authorised medicine and maintain standards, including postmarketing surveillance. In the absence of a marketing authorisation, a range of individual prescribers each has responsibility for off-label use, which would make it very difficult to develop and maintain a safety registry; pharmacists carry out a lot of work behind the scenes to support off-label medicines. A marketing authorisation provides an income stream that increases the justification for acceptance.
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secured funds from the European Commission’s Framework 
eyity off-patent medicines and the authors have successfully 
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WHAT SHOULD THE DRUG DEVELOPMENT PLAN LOOK LIKE?
One approach is illustrated in figure 1. This represents a balance 
timey research and filling in gaps in knowledge.

CONCLUSIONS
Azithromycin is an attractive medicine to evaluate for the pre-
vention of CLD. Epidemiological work, research in animals 
and early phase clinical trials in humans support the need 
for further investigation of this possible use. To date, it has a 
favourable safety profile and preliminary PK studies have been 
completed. Rational drug development will require an inte-
grated consideration of pharmaceutical quality, dosage regi-
men, efficacy and safety. Medicines research can no longer be 
curiosity driven but must be based on a careful line of thinking 
about formulation, optimised dosage regimen, clinical trials 
that explore the therapeutic possibility of the medicine and 
clinical trials that confirm the efficacy of the medicine.

Funding The authors are part of the EU funded FP7 programme TINN2.

Competing interests None.

Provenance and peer review Commissioned; externally peer reviewed.

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Figure 1  Sample drug development plan. DSMB, data and safety monitoring board; PD, pharmacodynamics; PK, pharmacokinetics; 
SUSAR suspected unexpected serious adverse reaction.
Arch Dis Child 2012; 97: 573–577. doi:10.1136/adc.2010.195180


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Arch Dis Child 2012 97: 573-577 originally published online June 22, 2011
doi: 10.1136/adc.2010.195180

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