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To cite this article: JoonHo Lee, Roberto Romero, Sun Min Kim, Piya Chaemsaithong, Chan-Wook Park, Joong Shin Park, Jong Kwan Jun & Bo Hyun Yoon (2016) A new anti-microbial combination prolongs the latency period, reduces acute histologic chorioamnionitis as well as funisitis, and improves neonatal outcomes in preterm PROM, The Journal of Maternal-Fetal & Neonatal Medicine, 29:5, 707-720, DOI: 10.3109/14767058.2015.1020293

To link to this article: http://dx.doi.org/10.3109/14767058.2015.1020293

Published online: 16 Sep 2015.

Article views: 390
A new anti-microbial combination prolongs the latency period, reduces acute histologic chorioamnionitis as well as funisitis, and improves neonatal outcomes in preterm PROM

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Abstract

Objective: Antibiotic administration is a standard practice in preterm premature rupture of membranes (PROM). Specific anti-microbial agents often include ampicillin and/or erythromycin. Anaerobes and genital mycoplasmas are frequently involved in preterm PROM, but are not adequately covered by antibiotics routinely used in clinical practice. Our objective was to compare outcomes of PROM treated with standard antibiotic administration versus a new combination more effective against these bacteria.

Study design: A retrospective study compared perinatal outcomes in 314 patients with PROM <34 weeks receiving anti-microbial regimen 1 (ampicillin and/or cephalosporins; n = 195, 1993–2003) versus regimen 2 (ceftriaxone, clarithromycin and metronidazole; n = 119, 2003–2012). Intra-amniotic infection/inflammation was assessed by positive amniotic fluid culture and/or an elevated amniotic fluid MMP-8 concentration (>23 ng/mL).

Results: (1) Patients treated with regimen 2 had a longer median antibiotic-to-delivery interval than those with regimen 1 (median interquartile range 23 d (10–51 d) versus 12 d (5–52 d), p < 0.001); (2) patients who received regimen 2 had lower rates of acute histologic chorioamnionitis (50.5% versus 66.7%, p < 0.05) and funisitis (13.9% versus 42.9%, p < 0.0001) than those who had received regimen 1; (3) the rates of intra-ventricular hemorrhage (IVH) and cerebral palsy (CP) were significantly lower in patients allocated to regimen 2 than regimen 1 (IVH: 2.1% versus 19.0%, p < 0.001 and CP: 0% versus 5.7%, p < 0.05); and (4) subgroup analysis showed that regimen 2 improved perinatal outcomes in pregnancies with intra-amniotic infection/inflammation, but not in those without intra-amniotic infection/inflammation (after adjusting for gestational age and antenatal corticosteroid administration).

Conclusion: A new antibiotic combination consisting of ceftriaxone, clarithromycin, and metronidazole prolonged the latency period, reduced acute histologic chorioamnionitis/funisitis, and improved neonatal outcomes in patients with preterm PROM. These findings suggest that the combination of anti-microbial agents (ceftriaxone, clarithromycin, and metronidazole) may improve perinatal outcome in preterm PROM.

Keywords

Ceftriaxone, cerebral palsy, clarithromycin, intra-amniotic infection, intra-amniotic inflammation, intra-ventricular hemorrhage, metronidazole, preterm birth

Introduction

Preterm premature rupture of membranes (PROM) complicates 2% of all pregnancies [1–5] and accounts for approximately one-third of all cases of preterm birth [6]. Preterm PROM has been the subject of several clinical and epidemiologic studies [7–25], and is considered one of the “great obstetrical syndromes” [26–28] responsible for spontaneous preterm birth [26,29,30]. Microbial invasion of the amniotic cavity (MIAC) is detected in approximately 30% of affected patients when using cultivation techniques [31–54], and in 50% when using a combination of cultivation and molecular techniques [55–60]. Studies in which serial
amniocenteses have been performed in patients with preterm PROM indicate that the prevalence of MIAC increases over time, and reaches approximately 75% when patients with preterm PROM begin preterm labor [36]. Thus, infection of the amniotic cavity seems to play a role in both the genesis of preterm PROM [31–64] and the onset of preterm labor [36]. Hence, the standard of care is to administer antibiotics to patients with preterm PROM with the goal of treating or preventing ascending infection [1,65–81].

Randomized clinical trials [82–96], as well as several systematic reviews and meta-analyses [2,97–100], show that antimicrobial agents can prolong the latency period [2,83–89,91–94,97,100] and reduce the rates of clinical chorioamnionitis [2,22,83,86,89,90,92,94,97], neonatal morbidity (including neonatal infection) [2,82–84,86,90–92,94,97,98], major cerebral abnormalities identified by ultrasound, [2,83,92,94] and the need for surfactant administration and oxygen therapy [2,84,92,94]. While several antibiotic regimens have been proposed for preterm PROM, the combination of ampicillin/amoxicillin with erythromycin is among the most widely used [92,101]. Yet, a long-term follow-up study of infants included in the ORACLE trial showed that there was no demonstrable benefit or harm at age 7 with antibiotic administration to patients with preterm PROM [102]. Thus, questions remain about the optimal care for women presenting with preterm PROM.

Ampicillin and erythromycin are inadequate in eradicating many of the organisms detected in preterm PROM. This has been attributed to the limited transplacental passage, and hence suboptimal anti-microbial activity in the amniotic fluid (only 3% of erythromycin [103,104] and 2.6% of azithromycin [105] cross the placenta). Clarithromycin, a semi-synthetic macrolide, on the other hand, has a much higher rate of transplacental passage than erythromycin, and is effective for the treatment of genital mycoplasmas [106,107]. This has led to the proposal that clarithromycin could be useful in the setting of preterm PROM [106]. Other anti-microbial agents that might be helpful include metronidazole, which is particularly effective against anaerobic bacteria [108–110] involved in bacterial vaginosis [111–139] and preterm PROM [55,57,119,140–142], and ceftriaxone, which has a long half-life and has enhanced coverage for Gram-negative bacteria [143–145].

Based on our previous studies about the microbiology of preterm PROM, the reports about the enhanced placental transfer of clarithromycin [106,107], the difficulty in eradicating Ureaplasmas [43,57,171–180], with erythromycin [146–150], and evidence that frequently used anti-microbial agents do not eradicate or prevent MIAC in preterm PROM [151], we changed the clinical management of preterm PROM at our center to include the use of a combination of ceftriaxone, clarithromycin and metronidazole. Herein, we explore whether women who presented with preterm PROM after this change in practice had better or worse pregnancy outcomes than those who received the prior regimen (ampicillin or cephalosporins were the most commonly used).

Materials and methods

Study design

This was a retrospective cohort study which included women with singleton gestations admitted to the Seoul National University Hospital between January 1993 and June 2012 with the diagnosis of preterm PROM (<34 weeks of gestation). Amniocenteses is routinely offered for microbiologic studies and assessment of fetal lung maturity to all patients with the diagnosis of preterm PROM. The inclusion criteria were: (1) antenatal antibiotic treatment for at least 24 h, (2) availability of perinatal outcomes, and (3) availability of amniotic fluid obtained by transabdominal amniocentesis for microbiologic studies. Amniocenteses were performed after written informed consent was obtained. Rupture of membranes (ROM) was diagnosed by a previous history of watery vaginal discharge and a combination of the following tests: confirming leakage of amniotic fluid from cervical os, vaginal pooling of amniotic fluid, and a positive nitrazine test through a sterile speculum examination. The Institutional Review Board of the Seoul National University Hospital approved the collection and the use of samples and clinical information for research purposes. The Seoul National University Hospital has a Federal Wide Assurance with the Office for Human Research Protection of the Department of Health and Human Services of the United States.

Antibiotics

Between January 1993 and August 2003, patients (n = 195) received ampicillin and/or cephalosporins. Some patients (n = 33) received erythromycin (n = 23), metronidazole (n = 15), azithromycin (n = 8) or gentamicin (n = 1) in addition to ampicillin or cephalosporins [this regimen will be considered number 1 for the purpose of this manuscript (n = 195)]. Between September 2003 and June 2012, the antimicrobial regimen included ceftriaxone 1 g (intravenous) every 24 h, clarithromycin 500 mg (oral) every 12 h, and metronidazole (intravenous) 500 mg every 8 h, routinely administered to the patients with preterm PROM [regimen 2 (n = 119)]. Antibiotics were administered until the patient delivered or there was no evidence of amniotic fluid leakage, with the exception of metronidazole, which was administered for a maximum of 4 weeks. The study population was divided into two groups according to the antibiotic regimen (1 versus 2). Group B streptococcus (GBS) screening and intra-partum treatment has never been used in our institution because neonatal GBS sepsis is extremely rare.

Amniotic fluid

Amniotic fluid was cultured for aerobic and anaerobic bacteria and genital mycoplasmas using methods previously described [40,152–154]. An aliquot of amniotic fluid was examined in a hemocytometer chamber to determine the white blood cell count. Amniotic fluid not used for diagnostic tests was centrifuged and stored at −80°C.

Diagnosis of intra-amniotic infection/inflammation, acute histologic chorioamnionitis, funisitis, and perinatal outcomes

Intra-amniotic inflammation was defined as an elevated amniotic fluid matrix metalloproteinase-8 (MMP-8) concentration (>23 ng/mL), as previously reported [43,155–161]. The concentration of MMP-8 was measured in stored
amniotic fluid using a commercially available enzyme-linked immunosorbent assay (Amersham Pharmacia Biotech, Inc., Bucks, UK), according to the manufacturer’s instructions. The sensitivity of the test is 0.3 ng/mL while the intra- and inter-assay coefficients of variation are less than 10%. Intra-amniotic infection/inflammation was defined as a positive amniotic fluid culture and/or an elevated amniotic fluid MMP-8 concentration (>23 ng/mL) [43,155–161].

The diagnosis of acute histologic chorioamnionitis was made on the basis of the presence of acute inflammatory changes in the examination of the extra-placental chorioamnionic membrane roll and/or chorionic plate of the placenta [162–164]. Funisitis was diagnosed as the neutrophil infiltration into the umbilical vessel walls or Wharton’s jelly with the criteria reported previously [156,163–167]. Perinatal outcomes included pregnancy outcomes such as impending spontaneous preterm delivery and spontaneous preterm delivery, and neonatal outcomes such as respiratory distress syndrome (RDS), bronchopulmonary dysplasia (BPD), proven congenital neonatal sepsis, necrotizing enterocolitis (NEC), intra-ventricular hemorrhage (IVH), periventricular leukomalacia (PVL), and cerebral palsy (CP). Briefly, RDS was diagnosed as the presence of respiratory distress, an increased oxygen requirement (FiO2 >0.4), and diagnostic radiological and laboratory findings in the absence of any other causes of respiratory distress [168]. BPD was diagnosed using the criteria of the National Institute of Child Health Workshop definition, i.e., the treatment with oxygen >21% for at least 28 d, and also diagnosed in the presence of typical findings at autopsy [154]. Congenital neonatal sepsis was diagnosed in the presence of a positive-blood culture result within 72 h of delivery [165]. NEC was diagnosed in the presence of abdominal distension and feeding intolerance (vomiting or increased gastric residual) for at least 24 h with clear evidence of intramural air, perforation, and meconium plug syndrome by radiological examination, or definite surgical or autopsy findings of NEC [162]. IVH was diagnosed by ultrasonographic examination or magnetic resonance imaging (MRI) of the neonatal head (≥Grade II) [169]. PVL was diagnosed as the presence of cystic lesions within the peri-ventricular white matter by ultrasonographic examination or MRI. CP was diagnosed in the presence of definite abnormalities on the neurodevelopmental assessment (i.e., abnormalities of developmental milestones, posture evaluated by the Vojta method and reflex) and persistent abnormalities of muscle tone [169,170]. Composite neonatal morbidity was defined when one or more neonatal outcomes including RDS, BPD, congenital neonatal sepsis, NEC, and IVH were diagnosed.

Statistical analysis
For continuous variables, median and interquartile were calculated and Mann–Whitney U tests were employed. For categorical variables, frequencies and percentages were calculated and the χ² test or Fisher’s exact test were used. The generalized Wilcoxon test for survival analysis was used to compare the antibiotic-to-delivery interval between groups. Patients who were delivered because of maternal–fetal indications had their antibiotic-to-delivery interval considered as censored observations, with a censoring time equal to the antibiotic-to-delivery interval. To adjust for the gestational age at the time of the initiation of antibiotics therapy and intra-amniotic infection/inflammation, logistic regression analysis and Cox proportional hazards modeling were used. Subgroup analysis according to the presence or absence of intra-amniotic infection/inflammation was also conducted to evaluate the efficacy of prophylactic antibiotic administration in the absence of intra-amniotic infection/inflammation and therapeutic antibiotic use in the presence of intra-amniotic infection/inflammation. Statistical analyses were conducted using SPSS Version 19.0 (SPSS Inc., Chicago, IL). All p values were two-sided and a p value of <0.05 was considered statistically significant.

Results

Characteristics of the study population

Three hundred and fourteen patients with preterm PROM were included in this study. Table 1 shows the demographic and clinical characteristics of the study population. The median gestational age at the time of the initiation of antibiotic therapy and amniocentesis was lower in patients who received antibiotic regimen 2 (ceftriaxone, clarithromycin, and metronidazole) than in those who received regimen 1 (p < 0.001, for each). Patients who received regimen 2 had a higher rate of intra-amniotic infection/inflammation and a higher median amniotic fluid MMP-8 concentration than in those who received regimen 1 (p < 0.001 for each).

Pregnancy and neonatal outcomes

Patients treated with regimen 2 had a significantly longer median antibiotic-to-delivery interval than those who received regimen 1 [median (interquartile) 23 d (10–51 d) versus 12 d (5–52 d), p < 0.01] (see Figure 1). For this analysis, 99 patients who delivered because of maternal and/or fetal indications had this interval censored. Multivariate survival analysis demonstrated that the antibiotic-to-delivery interval in patients who received regimen 2 was significantly longer than in those treated with regimen 1 after adjusting for gestational age at the time of the initiation of antibiotics therapy, the presence of intra-amniotic infection/inflammation, and antenatal corticosteroid administration (hazards ratio 0.69, 95% confidence interval (CI) 0.49–0.96; p < 0.05).

Table 2 shows pregnancy outcomes according to antibiotic regimen. The rate of spontaneous preterm delivery within 7 d after initiation of anti-microbial therapy was lower in patients who received regimen 2 than in those who received regimen 1 [20.2% (21/104) versus 43.6% (78/179), p < 0.001], which remained significant after adjusting for gestational age at the time of the initiation of antibiotic treatment, the presence of intra-amniotic infection/inflammation, and antenatal corticosteroid administration (OR 0.40, 95% CI 0.21–0.77, p < 0.01). There was no difference in the rate of spontaneous preterm delivery before 34 weeks between the two groups. However, the rate of spontaneous preterm delivery before 32 weeks in patients who received regimen 2 was significantly lower than in those who received regimen 1 after adjusting for
Table 1. Demographics and clinical characteristics of the study population.

<table>
<thead>
<tr>
<th></th>
<th>Regimen 1 group* (n = 195)</th>
<th>Regimen 2 group† (n = 119)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (year)‡</td>
<td>30 (27–53)</td>
<td>32 (29–36)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Nulliparity (%)</td>
<td>47.2 (92/195)</td>
<td>54.6 (65/119)</td>
<td>NS</td>
</tr>
<tr>
<td>Prior preterm birth (%)</td>
<td>14.9 (29/195)</td>
<td>11.8 (14/119)</td>
<td>NS</td>
</tr>
<tr>
<td>Amniotic fluid MMP-8 &gt; 23 ng/mL (%)</td>
<td>41.0 (80/195)</td>
<td>61.3 (73/119)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Positive amniotic fluid culture (%)</td>
<td>23.1 (45/195)</td>
<td>28.7 (33/115)</td>
<td>NS</td>
</tr>
<tr>
<td>Intra-amniotic fluid MMP-8 (ng/mL)†</td>
<td>7.1 (0.9–161.0)</td>
<td>46.3 (7.9–242.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gestational age at the time of the initiation of antibiotic treatment (weeks)‡</td>
<td>45.6 (89/195)</td>
<td>66.4 (79/119)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gestational age at amniocentesis (weeks)†</td>
<td>30.1 (27.1–32.6)</td>
<td>27.7 (23.4–31.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Amniotic fluid MMP-8 (%)</td>
<td>7.1 (0.9–161.0)</td>
<td>46.3 (7.9–242.7)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

MMP-8, matrix metalloproteinase-8; NS, not significant.
*Regimen 1: ampicillin and/or cephalosporins, and in some patients other antibiotics were combined with them.
†Regimen 2: ceftriaxone, clarithromycin, and metronidazole
‡Median (interquartile).

Figure 1. Survival analysis of the antibiotic-to-delivery interval according to the antibiotic regimen. Patients who received regimen 2 (ceftriaxone, clarithromycin, and metronidazole) had a significantly longer median antibiotics-to-delivery interval than those who received regimen 1 (ampicillin and/or cephalosporins, in some patients other antibiotics were combined with them.) median (interquartile) 23 d (10–51 d) versus 12 d (5–52 d), p < 0.01, which remained significant after adjusting for gestational age at the time of the initiation of antibiotic therapy, the presence of intra-amniotic infection/inflammation, and antenatal corticosteroid administration (hazards ratio 0.69, 95% (CI) 0.49–0.96; p <0.05 by Cox proportional hazards model analysis).

Intra-amniotic infection/inflammation and antibiotic regimen

As intra-amniotic infection/inflammation is one of the most important and well-established risk factors for adverse pregnancy and neonatal outcomes, subgroup analysis according to the presence or absence of intra-amniotic infection/inflammation was performed. Tables 4 and 5 show pregnancy and neonatal outcomes in patients with and without intra-amniotic infection/inflammation. Among patients without intra-amniotic infection/inflammation, there were no differences in outcomes between the two antibiotic regimens.

In contrast, among patients with intra-amniotic infection/inflammation, the rates of spontaneous preterm delivery within 14 d, 7 d, and 2 d after initiation of antibiotic treatment were significantly lower in those receiving regimen 2 than in those receiving regimen 1 after adjusting for gestational age at the time of initiation of antibiotic treatment and antenatal corticosteroid administration (for within 14 d: OR 0.17, 95% CI 0.07–0.41; for within 7 d: OR 0.22, 95% CI 0.09–0.50; for within 2 d: OR 0.24, 95% CI 0.08–0.69) (p <0.01, for each). The rates of spontaneous preterm delivery before 34 weeks and 32 weeks of gestation were also lower in patients treated with regimen 2 than in those treated with regimen 1 after adjusting for confounding factors (for within 34 weeks: OR 0.18, 95% CI 0.05–0.62; for 32 weeks before 34 weeks: OR 0.11, 95% CI 0.03–0.44) (p <0.01, for each). Acute histologic chorioamnionitis and funisitis were observed less frequently in patients receiving regimen 2 than in those receiving regimen 1 (for acute histologic chorioamnionitis: OR 0.08, 95% CI 0.03–
0.27; for funisitis OR 0.08, 95% CI 0.03–0.20 (p < 0.001, for each). Patients who received antibiotic regimen 2 had lower rates of IVH, PVL, and composite neonatal morbidity and a higher survival rate than those who had received regimen 1 after adjusting for confounding factors, including gestational age at the time of the initiation antibiotic treatment and antenatal corticosteroid administration (for IVH: OR 0.06, 95% CI 0.01–0.31; for PVL: OR 0.07, 95% CI 95% CI 0.01–0.85; for composite neonatal morbidity: OR 0.24, 95% CI 0.01–0.62; for survival: OR 3.63, 95% CI 1.12–11.79) (p < 0.05, for each). There was no case of CP (0/59) in those who received regimen 2, while CP was diagnosed in those who received regimen 1 (p < 0.05).

A significantly longer antibiotic-to-delivery interval was observed in patients with intra-amniotic infection/inflammation who received antibiotic regimen 2 than in those who received antibiotic regimen 1 (see Figure 2) [median (interquartile) 29 d (10–60 d) versus 5 d (2–14 d), p < 0.001], but not in patients without intra-amniotic infection/inflammation (p > 0.4). Multivariate survival analysis also demonstrated that the antibiotic-to-delivery interval in patients who received antibiotic regimen 2 was significantly longer than in those who received antibiotic regimen 1 after adjusting for gestational age at the time of initiation of antibiotic treatment and antenatal corticosteroid administration, only in the context of intra-amniotic infection/inflammation (hazards ratio 0.41, 95% CI 0.27–0.63; p < 0.001).

**Discussion**

**Principal findings of this study**

The administration of ceftriaxone, clarithromycin, and metronidazole (regimen 2), when compared to that of regimen 1 (mainly ampicillin and/or cephalosporins), in patients with preterm PROM was associated with: (1) a significantly longer latency period and lower rates of spontaneous preterm delivery within 7 d; (2) lower rates of acute histologic...
Table 4. Perinatal outcomes of patients without intra-amniotic infection/inflammation according to the regimen of antibiotics used.

<table>
<thead>
<tr>
<th>Regimen 1 group* (n = 106)</th>
<th>Regimen 2 group† (n = 40)</th>
<th>p value[ and odds ratio (OR) and 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at amniocentesis (weeks)|$</td>
<td>31.4 (28.6–32.8)</td>
<td>31.2 (28.3–32.9)</td>
</tr>
<tr>
<td>Gestational age at the time of the initiation of antibiotics treatment (weeks)|$</td>
<td>31.4 (28.5–32.8)</td>
<td>31.2 (28.3–32.9)</td>
</tr>
<tr>
<td>Gestational age at delivery (weeks)|$</td>
<td>34.3 (32.9–37.2)</td>
<td>33.5 (31.8–34.1)</td>
</tr>
<tr>
<td>Spontaneous preterm delivery (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;37 weeks</td>
<td>67.1 (57/85)</td>
<td>92.3 (24/26)</td>
</tr>
<tr>
<td>&lt;34 weeks</td>
<td>37.1 (36/97)</td>
<td>48.5 (16/33)</td>
</tr>
<tr>
<td>&lt;32 weeks|$</td>
<td>23.2 (13/56)</td>
<td>28.6 (6/21)</td>
</tr>
<tr>
<td>Spontaneous delivery (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤14 d</td>
<td>40.6 (39/96)</td>
<td>43.8 (14/32)</td>
</tr>
<tr>
<td>≤7 d</td>
<td>29.0 (29/100)</td>
<td>25.0 (9/36)</td>
</tr>
<tr>
<td>≤2 d</td>
<td>6.6 (7/106)</td>
<td>12.8 (5/39)</td>
</tr>
<tr>
<td>Acute histologic chorioamnionitis (%)</td>
<td>44.6 (37/83)</td>
<td>38.2 (13/34)</td>
</tr>
<tr>
<td>Funisitis (%)</td>
<td>22.6 (19/84)</td>
<td>11.1 (4/36)</td>
</tr>
<tr>
<td>Respiratory distress syndrome (%)</td>
<td>8.2 (8/98)</td>
<td>10.5 (4/38)</td>
</tr>
<tr>
<td>Bronchopulmonary dysplasia (%)</td>
<td>4.1 (4/97)</td>
<td>7.9 (3/38)</td>
</tr>
<tr>
<td>Congenital neonatal sepsis, proven (%)</td>
<td>4.1 (4/98)</td>
<td>0.0 (0/37)</td>
</tr>
<tr>
<td>Intra-ventricular hemorrhage (%)</td>
<td>8.3 (8/96)</td>
<td>0.0 (0/37)</td>
</tr>
<tr>
<td>Peri-ventricular leukomalacia (%)</td>
<td>2.1 (2/96)</td>
<td>0.0 (0/38)</td>
</tr>
<tr>
<td>Necrotizing enterocolitis (%)</td>
<td>2.1 (2/97)</td>
<td>10.5 (4/38)</td>
</tr>
<tr>
<td>Composite neonatal morbidity (%)</td>
<td>18.4 (18/98)</td>
<td>23.7 (9/38)</td>
</tr>
<tr>
<td>Cerebral palsy (%)</td>
<td>9.2 (9/98)</td>
<td>10.5 (4/38)</td>
</tr>
<tr>
<td>Necrotizing enterocolitis (%)</td>
<td>2.1 (2/97)</td>
<td>10.5 (4/38)</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>96.0 (96/100)</td>
<td>94.9 (37/39)</td>
</tr>
</tbody>
</table>

Table 5. Perinatal outcomes of patients with intra-amniotic infection/inflammation according to the regimen of antibiotics used.

<table>
<thead>
<tr>
<th>Regimen 1 group* (n = 89)</th>
<th>Regimen 2 group† (n = 40)</th>
<th>p value[ and odds ratio (OR) and 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at amniocentesis (weeks)|$</td>
<td>29.4 (26.5–32.0)</td>
<td>26.1 (23.0–28.9)</td>
</tr>
<tr>
<td>Gestational age at the time of the initiation of antibiotics treatment (weeks)|$</td>
<td>28.9 (26.1–32.0)</td>
<td>25.1 (22.6–28.4)</td>
</tr>
<tr>
<td>Gestational age at delivery (weeks)|$</td>
<td>30.4 (27.5–32.9)</td>
<td>28.6 (25.4–32.6)</td>
</tr>
<tr>
<td>Spontaneous preterm delivery (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;37 weeks</td>
<td>100.0 (71/71)</td>
<td>97.8 (45/46)</td>
</tr>
<tr>
<td>&lt;34 weeks</td>
<td>91.5 (65/71)</td>
<td>77.8 (42/54)</td>
</tr>
<tr>
<td>&lt;32 weeks|$</td>
<td>89.7 (52/58)</td>
<td>70.5 (31/44)</td>
</tr>
<tr>
<td>Spontaneous delivery (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤14 d</td>
<td>83.6 (61/73)</td>
<td>36.7 (22/60)</td>
</tr>
<tr>
<td>≤7 d</td>
<td>62.0 (49/79)</td>
<td>71.6 (16/28)</td>
</tr>
<tr>
<td>≤2 d</td>
<td>29.8 (25/84)</td>
<td>6.7 (5/75)</td>
</tr>
<tr>
<td>Acute histologic chorioamnionitis (%)</td>
<td>92.9 (65/70)</td>
<td>57.1 (36/63)</td>
</tr>
<tr>
<td>Funisitis (%)</td>
<td>67.1 (47/70)</td>
<td>15.4 (10/65)</td>
</tr>
<tr>
<td>Respiratory distress syndrome (%)</td>
<td>14.7 (11/75)</td>
<td>18.0 (11/61)</td>
</tr>
<tr>
<td>Bronchopulmonary dysplasia (%)</td>
<td>25.0 (17/68)</td>
<td>41.4 (24/58)</td>
</tr>
<tr>
<td>Congenital neonatal sepsis, proven (%)</td>
<td>5.3 (4/76)</td>
<td>1.6 (1/61)</td>
</tr>
<tr>
<td>Intra-ventricular hemorrhage (%)</td>
<td>33.3 (24/72)</td>
<td>3.3 (2/60)</td>
</tr>
<tr>
<td>Peri-ventricular leukomalacia (%)</td>
<td>11.6 (8/70)</td>
<td>1.7 (1/60)</td>
</tr>
<tr>
<td>Necrotizing enterocolitis (%)</td>
<td>5.4 (4/74)</td>
<td>8.3 (5/60)</td>
</tr>
<tr>
<td>Composite neonatal morbidity (%)</td>
<td>57.5 (42/73)</td>
<td>45.0 (27/60)</td>
</tr>
<tr>
<td>Cerebral palsy (%)</td>
<td>11.1 (7/63)</td>
<td>0.0 (0/59)</td>
</tr>
<tr>
<td>Survival (%)</td>
<td>79.0 (64/81)</td>
<td>76.3 (58/76)</td>
</tr>
</tbody>
</table>
Figure 2. Survival analysis of the antibiotic-to-delivery interval according to the antibiotic regimen in patients with intra-amniotic infection/inflammation at the time of amniocentesis. A significant longer antibiotic-to-delivery interval was observed in patients with intra-amniotic infection/inflammation who received antibiotic regimen 2 (ceftriaxone, clarithromycin, and metronidazole) than in those who received antibiotic regimen 1 (ampicillin and/or cephalosporins, in some patients other antibiotics were combined with them) [median (interquartile) 29 d (10–60 d) versus 5 d (2–14 d), p < 0.001]. Multivariate survival analysis using the Cox proportional hazards model analysis also demonstrated the longer antibiotic-to-delivery interval in patients who received antibiotic regimen 2 than in those who received regimen 1 after adjusting for gestational age at the time of initiation of antibiotic treatment and antenatal corticosteroid administration (hazards ratio 0.41, 95% CI 0.27–0.63; p < 0.001).

Microorganisms involved in preterm PROM

Thirty to 50 percent of patients with preterm PROM have MIAC, detected using a combination of cultivation [31–54] and molecular microbiologic techniques [55–60]. The organisms involved included Tenericutes such as *Ureaplasma*, *Mycoplasma hominis* [43,57,171–180], *Haemophilus* sp., *Streptococcus agalactiae*, GBS, *Streptococcus salivarius*, *Streptococcus pneumonia*, *Enterococcus faecalis*, or *Lactobacillus* species), *Fusobacteria* (e.g. *Fusobacterium nucleatum*, *Neathia sp.*), *Bacteroidetes* (e.g. *Bacteroides fragilis*, *Bacteroides sp.*, *Prevotella sp.*), *Proteobacteria* (*Haemophilus sp.*), *Actinobacteria* (e.g. *Gardnerella vaginalis*, *Bifidobacterium sp.*, *Rothia sp.*), and *Candida* sp. [55,57,186]. Polymicrobial infections, which were present in 30% of cases, and fungi were found in 5% of cases [55,57]. Given the broad range of microorganisms involved and the difficulty in identifying the microorganism in most patients, broad anti-microbial coverage has been used in clinical trials, as well as in practice [101,187–189].

Antibiotic administration in patients with preterm PROM: randomized clinical trials, systematic reviews, and meta-analyses

A Cochrane review including 22 randomized controlled trials of antibiotic administration to patients with preterm PROM concluded that this intervention prolonged the latency period, neonatal infection, abnormal sonographic cerebral findings, reduced respiratory morbidities, and the need for oxygen and surfactant, and also reduced the frequency of clinical chorioamnionitis [2]. However, most patients comprising these meta-analyses came from two trials. The first, by Mercer et al. [92], used a combination of intravenous ampicillin and erythromycin for 2 d, followed by oral amoxicillin and erythromycin for 5 d. The second trial, by Kenyon et al. [94], used a factorial design with four treatment arms including oral anti-microbials for 10 d (the arms were: placebo, oral erythromycin, amoxicillin-clavulanic acid, or the combination of erythromycin and amoxicillin-clavulanic acid). Patients receiving erythromycin and/or amoxicillin-clavulanic acid remained undelivered at 48 h more frequently than patients in the placebo group. However, the use of amoxicillin-clavulanic acid did not yield additional benefits (i.e. reduction in neonatal morbidity) observed in patients allocated to receive erythromycin [94]. In the study of Kenyon et al., but not in that by Mercer et al., NEC was more frequent among patients receiving amoxicillin-clavulanic acid [94]. The findings of the study of Mercer et al. [92] and those of Kenyon et al. [94] have informed practice in the United States and Europe. A 7-year follow-up study of the ORACLE trial has not shown a benefit of antibiotic administration in terms of CP, behavioral problems, or childhood death [102]. Therefore, the benefits of antibiotic administration are demonstrable only in short-term outcomes so far.

The rationale for a new antibiotic regimen in preterm PROM

Isolated reports document that the eradication of MIAC in preterm PROM is possible [190–194]. However, in a study in which amniocentesis was performed before and after the administration of antibiotics, these agents did not eradicate or prevent subsequent intra-amniotic infection [151]. Potential explanations for these observations are that some antibiotics have poor transplacental passage (i.e. erythromycin) [103,104] and that infections may develop from organisms not adequately covered by the anti-microbials [146–150]. For example, a recent study indicated that 80% of genital mycoplasmas are resistant to erythromycin: this is largely the case for *Ureaplasma parvum*, the most common biovar in the amniotic fluid of our patients [195]. This was the impetus for considering a new antibiotic regimen in our practice. Oral clarithromycin was chosen because of its efficacy against...
mycoplasmas and greater transplacental passage than erythromycin and azithromycin [103,104]. Indeed, clarithromycin has a lower minimal inhibitory concentration (MIC) for Ureaplasma than erythromycin [196,197] that has greater anti-microbial properties against Ureaplasma [198]. A previous study demonstrated that the bioavailability of clarithromycin after oral administration is sufficient for adequate anti-microbial activity [199,200]. Moreover, the concentration of an active metabolite, 14-hydroxyclarithromycin, in plasma is greater after oral administration than following intravenous infusion [199]. Intravenous metronidazole was included because of its powerful effect against anaerobic bacteria frequently present in preterm PROM [108,109]. Intravenous administration was chosen over oral administration, hoping to decrease the likelihood of gastrointestinal side effects associated with oral use [108]. A third-generation cephalosporin, intravenous ceftriaxone, was included to enhance coverage of aerobic organisms such as Streptococcus sp., Haemophilus sp., and beta-lactamase-producing strains of Hemophilus sp. [143–145]. Moreover, ceftriaxone readily crosses the placenta and can be found in umbilical cord blood, amniotic fluid, and the placenta. In these biological fluids and tissue, the concentrations achieved are sufficient for anti-microbial effects to be obtained [201]. Antibiotics were administered until delivery, unless the patient remained undelivered for 4 weeks (in which case metronidazole was discontinued because of the concern for adverse events). The rationale for continuing antibiotics until delivery was that intra-amniotic infection can occur despite short-term antibiotic administration [151]. The practice at the Seoul National University Hospital had been to administer antibiotics from the time of preterm PROM until delivery. Thus, the main difference between regimens 1 and 2 was the spectrum of antibiotic coverage rather than the duration of treatment.

An expanded anti-microbial spectrum of treatment resulted in improved pregnancy and neonatal outcomes

The results of the current study indicated that the new antibiotic regimen was associated with a longer latency period, lower rates of spontaneous preterm delivery within 48 h, 7 d, and 14 d as well as improvement of neonatal outcomes and a reduction of the rates of acute histologic chorioamnionitis and funisitis. This benefit occurred in patients with demonstrable intra-amniotic infection/inflammation at the beginning of the therapy, but not in patients without this condition. Our findings are in contrast to those previously reported, indicating that a combination of amoxicillin and erythromycin administered for a short period of time did not reduce the frequency of acute histologic chorioamnionitis [96,202,203]. Of interest, in a randomized clinical trial in which the efficacy of cefazolin, cefazolin plus erythromycin, or cefazolin plus clarithromycin for 7 d was compared, the use of cefazolin with clarithromycin was associated with a reduction in the percentage of patients with severe funisitis. The lack of difference in neonatal outcomes in that study could be attributed to the sample size, as there were less than 36 patients per group [96].

A likely explanation for the beneficial effects of the new antibiotic regimen is the broad anti-microbial coverage. However, clarithromycin, which has immunomodulatory properties, has been used in experimental sepsis and acute pyelonephritis [204–210], and it was shown to inhibit the production of pro-inflammatory factors by human mononuclear cells [211–213]. The combination of ampicillin and erythromycin in patients with preterm PROM was previously shown to decrease the maternal concentration of granulocyte colony stimulating factor (G-CSF) but not of intercellular adhesion molecule (ICAM-1), interleukin-6 (IL-6), IL-10, and tumor necrosis factor-alpha (TNF-α) [189]. However, antibiotic administration did not decrease umbilical concentrations of cytokines or adhesion molecules [189]. Further studies are required to determine if the new anti-microbial combination can decrease the production of pro-inflammatory cytokines. The reduction in the frequency of funisitis suggests that this is likely to be the case.

Duration of anti-microbial therapy

The duration of anti-microbial therapy in preterm PROM has varied among studies [95,214,215]. Mercer et al. administered parenteral antibiotics for 2 d followed by oral administration for 5 d (total of 7 d) [92]. Kenyon et al. used the oral route for 10 d of treatment [94]. The results of these two studies are largely consistent on the short-term benefits for prolongation of the latency period, and reduction in neonatal infection rates.

The duration of anti-microbial therapy for most clinical infections is largely arbitrary and generally for 7 d. This has been the case for urinary tract infections [216–220], pyelonephritis [221–226], and endometritis [227–230]. However, recent studies have shown that a shorter duration of antibiotics in pregnant and non-pregnant subjects has similar efficacy and potential benefit in terms of compliance and cost [231–234]. Therefore, investigators have attempted to address whether the benefits of anti-microbial therapy can be achieved with a shorter duration. Segel et al. [214] compared the efficacy of the antibiotic administration for 3 d versus 7 d of intravenous ampicillin in patients with preterm PROM between 24 and 33 weeks of gestation. For the first 48 h, all patients received parenteral ampicillin (2 g IV every 6 h), then were randomized to oral therapy with ampicillin 500 mg per oral every 6 h for 3 d or 7 d. There were no statistically significant differences in achieving a 7-d latency period (RR 0.83; 95% 0.51–1.38) or in the rate of clinical chorioamnionitis, endometritis, or composite neonatal morbidity [214]. Subsequently, Lewis et al. [95] reported the result of a prospective randomized trial in which patients were allocated to receive either 3 d or 7 d of ampicillin plus sulbactam (intravenous 3 g every 6 h for either 12 or 28 doses) in patients with preterm PROM (24–34 weeks; n = 84 patients). There were no differences in the latency period between the two groups or the frequency of neonatal complications [95]. Collectively, these studies raised the question of whether 7 d of treatment is required. However, the statistical power to detect the difference is a potential limitation.

The other question about the duration of anti-microbial therapy is whether longer treatment can result in a beneficial effect. If the goal of treatment is to control the growth of microorganisms in the amniotic cavity or to prevent secondary intra-amniotic infection, then a longer duration of therapy has
potential benefits. A previous study indicated that when treatment with an anti-microbial agent was administered for a total of 7 d (ampicillin and erythromycin) or 10–14 d (ceftriaxone, clindamycin, and erythromycin), microbial eradication did not occur in a large number of patients and inflammation developed in 32% of cases that did not receive it at the time of the first amniocentesis [151]. This raised the question of whether a longer duration of anti-microbial therapy may be more efficacious. The practice at the Seoul National University Hospital for the last 25 years has been to administer the anti-microbial agent until delivery has occurred rather than stopping at 7 d or 10 d. Therefore, a unique feature of the current study is that antibiotics were administered for an extended period of time much longer than that conventionally used in the United States or Europe. Since the duration of antibiotic treatment was similar for regimens 1 and 2, this could not account for differences observed in the outcome of this study. Exposure to antibiotics could be associated with a short-term individual effect or community-wide consequences such as the emergence of resistant organisms similar to those that have been identified after the widespread utilization of GBS prophylaxis [235–237]. Our hospital did not detect an increase in the frequency of resistant organisms in the newborn intensive care unit or in the newborns treated in this study.

Strengths and limitations

The strength of this study is that it is the only systematic examination of the effect of a new antibiotic regimen in preterm PROM at a single institution in which the anti-microbial agents were used for an extended period of time rather than a standard short-term administration. Moreover, we were able to assess the intra-amniotic inflammatory state of the patients enrolled in the study, which allowed us to detect that anti-microbial agents prolong pregnancy and improve neonatal outcomes in patients with (but not without) demonstrable intra-amniotic infection/inflammation.

Several limitations must be acknowledged. First, this was not a randomized controlled trial, which is the gold standard to test interventions in clinical medicine. Second, the use of a historical control group has the potential to introduce bias, particularly in outcomes that could change over time. This may affect some neonatal endpoints, but would not affect the duration of the latency period or the rate of acute histologic chorioamnionitis and funisitis. It is noteworthy that the median gestational age at the time of the administration of antibiotics to patients who received regimen 1 was significantly higher than that of patients who received regimen 2 (30.1 weeks versus 27.7 weeks; p < 0.001). Interestingly, the frequency of intra-amniotic infection/inflammation was also greater in patients who received regimen 2 than in those who received regimen 1.

It is difficult to determine which antibiotics among ceftriaxone, clarithromycin, and metronidazole were responsible for better perinatal outcomes in regimen 2 compared to regimen 1. However, the combination of those three anti-biotics in the current study, having anti-microbial activity against most microorganisms found in the amniotic fluid of patients with preterm PROM and the greatest anti-microbial potency against genital mycoplasmas, could improve prenatal outcomes through the eradication of pre-existing intra-amniotic infection/inflammation, the prevention of de novo intra-amniotic infection/inflammation and the reduction of the frequency of funisitis, a histologic hallmark of fetal inflammatory response syndrome [166].

Conclusions

Antibiotic treatment with intravenous ceftriaxone, metronidazole, and oral clarithromycin (regimen 2) was associated with the prolongation of the duration of pregnancy after preterm PROM, reduce acute histologic chorioamnionitis and acute funisitis, as well as the rate of adverse neonatal outcomes compared to regimen 1. The findings herein justify further studies to determine the optimal anti-microbial regimen and duration of treatment in patients with preterm PROM.

Declaration of interest

The authors report no conflicts of interest. This research was supported by a grant of the Korean Health Technology R&D Project through the Korea Health Industry Development Institute (KHIDI), funded by the Ministry of Health & Welfare, Republic of Korea (grant number: HI12C0768). This research was also supported, in part, by the Perinatology Research Branch, Division of Intramural Research, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Department of Health and Human Services (NICHD/NIH/DHHS), U.S.A.

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