Persistent Bacteremia in Rabbit Fetuses despite Maternal Antibiotic Therapy in a Novel Intrauterine-Infection Model


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The effect of optimized maternal therapy by bactericidal agents was evaluated in a reproducible rabbit model of Escherichia coli maternofetal infection simulating human pharmacokinetics. Intravenous antibiotic therapy was begun in the pregnant rabbit 12 h after bacterial intrauterine inoculation, using a computer-controlled pump to simulate human pharmacokinetics of ceftriaxone (1 g/day) associated or not with gentamicin (3 mg/kg of body weight/day). Data were compared for fetal survival, quantitative blood cultures, fetal histology in treated versus untreated groups, and maternal and fetal antibiotic concentrations in plasma in treated animals. Antibiotic therapy led to dramatic improvement in maternal outcome (100% survival versus 100% death in the untreated group in association with maternal septicemia). Fetal survival also improved, with the two-drug combination providing a more potent effect. After 3 days of treatment, 32% of fetuses survived with one-drug therapy and 62% with two-drug therapy (Yates corrected $\chi^2$, $P < 0.05$). In untreated animals, bacterial counts in blood cultures increased rapidly during the first 24 h up to 8.1 ± 0.5 log CFU/ml, but remained relatively constant at all times with antibiotic treatment: 4.5 ± 0.7 log CFU/ml at the start of treatment and 6.2 ± 0.4 and 5.2 ± 0.9 log CFU/ml after 72 h for one- and two-drug therapy, respectively (data are means ± standard deviations). The failure of animals to be cured after 3 days of treatment was not due to an inadequate concentration of ceftriaxone, as the residual level in fetal serum at sacrifice was more than 1,000 times the MIC of the microbe. Unexpectedly, inflammation in fetal lung decreased in the treated group after as little as 24 h of antibiotic therapy, despite persistent bacteremia. Although maternal outcome improved and drug concentrations were above the MIC, the treatment did not achieve sterilization of fetuses in utero for this rabbit E. coli maternofetal infection. However, fetal survival showed some improvement, and the histologic features of lung inflammation were reduced.

Maternofetal infection is a frequent and severe pathology currently regarded as a public health problem, with an incidence of 1/1,000 births and a perinatal death rate of up to 20% for manifest forms of the disease. Maternofetal infection is considered to be the leading cause of death during the neonatal period, particularly because of the resulting prematurity (14, 34, 35). The strategy to adopt in cases of chorioamnionitis remains controversial, but physicians are likely either to continue the pregnancy under maternal antibiotic therapy or terminate it and treat the newborn ex utero, despite the risks related to extreme prematurity (13, 15, 17, 28).

When clinical studies are difficult to perform, notably because of ethical problems, an experimental approach provides an interesting and informative alternative for therapeutic assessment (5, 36). Various experimental models of perinatal infection have been reported, but many are actually concerned with immediate postnatal infection (20, 26, 31, 32). Although animal studies of prenatal infection are less common, they generally proved a beneficial effect of maternal antimicrobial therapy. Reduced rates of abortion and fetal death associated with endometrial and amniotic sterilization are usually achieved, particularly when treatment is begun immediately after inoculation (9, 12, 19, 27). However, a delay of only a few hours can seriously affect the rate of bacterial eradication (12, 19), casting doubt on the value of maternal antibiotic therapy for curative purposes. Moreover, bacteriologic failure raises various puzzling questions, notably because of a lack of fetal pharmacokinetic documentation and quantitative bacteriologic data.

To clarify these points, maternal antibiotic therapy was evaluated in a prenatal infection model based on the following specifications: (i) optimization of treatment through the use of antibiotic therapy known to be bactericidal, (ii) controlled administration of antibiotics to ensure effective simulation of human maternal plasma pharmacokinetics, (iii) measurement of residual plasma antibiotic concentrations in fetuses and mothers, and (iv) maternal and fetal bacteriologic assessment by quantitative blood cultures for more precise assessment of the therapeutic effect.

The purpose of this study was to determine whether optimized maternal antibiotic therapy (using an antibiotic association consistent with bacterial susceptibility testing and under pharmacokinetic control in the animal) could provide a cure for the infected fetus in utero.
MATERIALS AND METHODS

Microorganism. This study was performed with Escherichia coli K1, strain RS218 (O18:K1:HT), isolated from the cerebrospinal fluid of a newborn. Virulence factors and invasion genes have been characterized in this strain (2, 22). The MICs of ceftriaxone and gentamicin were 0.06 and 0.5 mg/liter, respectively.

The day before the experiment, the study strain was incubated in Mueller-Hinton (MH) broth (Difco, Becton Dickinson, Le Pont de Clain, France) for 24 h at 37°C. This suspension was then diluted in sterile NaCl 0.9% solution to obtain bacterial concentrations of 10^3, 10^6, or 10^8 CFU/ml, which were checked by nephelometry or quantitative cultures on MH agar (4).

Experimental model. Female New Zealand rabbits weighing 3.7 to 4.5 kg were obtained (CEGAV, Saint Marc d’Egrenne, France) 10 days before the end of their normal gestation period (i.e., 31 to 34 days) and provided ad libitum with water and food consisting of antibiotic-free granules. One week before the normal term of gestation (i.e., day 25), they were anesthetized with 25 mg of ketamine/kg. A 2-cm vertical incision was performed along the median line below the gravid uterus, and an inoculum of either 10^3, 10^6, or 10^8 CFU/ml was injected under visual control into one of the horns (intra-amniotic inoculation). The incision was then closed up level by level, and the animal was returned to its cage.

Treatment design. The mothers were randomly assigned to one of the following three groups: (i) untreated animals sacrificed 12 h (n = 5) or 24 h (n = 5) after inoculation or kept alive for follow-up (n = 5); (ii) animals treated by ceftriaxone alone for 24 h (n = 4), 48 h (n = 4) or 72 h (n = 4); (iii) or animals treated by ceftriaxone and gentamicin for 24 h (n = 3), 48 h (n = 4) or 72 h (n = 3).

In all cases, treatment was begun 12 h after intrauterine inoculation.

Antibiotic administration. Ceftriaxone (Rocphenine; Laboratoire Roche) and gentamicin (Gentamicine; Laboratoire Schering Plough) were diluted in a 0.9% NaCl solution for intravenous administration to mother rabbits through a catheter inserted into a marginal vein of the ear (for group iii each ear was used for an antibiotic). For both drugs, a computer-controlled variable infusion rate was delivered and adjusted, as previously reported (3, 18), in order to attain concentrations in mother rabbit plasma that mimicked the pharmacokinetics observed in humans after a single daily injection of ceftriaxone (1 g/day) or gentamicin (5 mg/kg/day). Accordingly, the objectives of this human pharmacokinetic simulation were a peak at 20 mg/liter and a half-life of 2 h for gentamicin and a peak at 150 mg/liter and a half-life of 8 h for ceftriaxone. The total doses actually administered to animals to meet these pharmacokinetic requirements were 315 and 16.6 mg/kg/day for ceftriaxone and gentamicin, respectively.

Pharmacokinetic study. Blood samples for antibiotic assays were obtained from mother rabbits (three or four mothers for each antibiotic) 24, 48, and 72 h after the beginning of treatment by means of a catheter inserted into the central artery of the ear contralateral to that infused with the antibiotic assayed. Fetal blood from three or four living fetuses per mother was sampled by intracardiac puncture at the time of sacrifice.

Ceftriaxone was assayed by high-performance liquid chromatography, based on the method proposed by Jelh et al. for beta-lactams (23), using a mobile phase comprising 0.03 M sodium phosphate (pH 1.9) (85% vol) and acetonitrile (15% vol), with a quantification limit of 1 mg/liter, a linearity range of up to 250 mg/liter, and interrun coefficient of variation at 9 mg/liter. Gentamicin concentrations were determined by fluorescence polarization immunoassay (24) (AxSYM; Abbott Laboratories, Rungis, France), with a detection threshold at 0.3 mg/liter, inter- and intrarun coefficients of variation below 5% within the working concentration range from 1 to 10 mg/liter, and automated dilution for samples with a higher concentration.

Judgment criteria. (i) Fetal survival. As echocardiographic evaluation of pre-natal survival was unsatisfactory in this model, true survival follow-up could not be performed. Thus, the fetuses were extracted by cesarean section under anesthesia (propofol [2 mg/kg]) to determine end-point survival rates 24, 48, or 72 h after the beginning of treatment, according to the study design described above. All animals were then sacrificed (ether for fetuses and 100 mg of thiopental sodium for mothers) to evaluate other judgment criteria. Untreated animals underwent cesarean section according to the same protocol 12 h after inoculation.

(ii) Maternal and fetal quantitative blood cultures. At the time of sacrifice, blood was withdrawn from the hearts of mothers and fetuses for quantitative cultures. The 100-μl blood samples were mixed with 500 μl of heparinized (5,000 IU/liter) physiological serum. After centrifugation (20 × g for 5 min), 100 μl of the pellet was plated on MH agar (Difco, Becton Dickinson) and incubated at 37°C for 24 h before bacterial counts were performed. To avoid any carryover phenomenon, blood was cultured in the presence of cephalexin and Enterococcus cloacae (P4524; Sigma, Lyon, France) at a dose of 0.72 IU/ml. This method allows detection of an inoculum of 10^6 CFU/ml.

(iii) Placental cultures. The placentas of each fetoplacental unit was rinsed with sterile saline and placed individually in MH broth containing cephalosporinase (0.72 IU/ml) of E. cloacae (P4524; Sigma) for 24-h incubation at 37°C. All tubes with turbid culture medium were then cultured again in specific esoin-methylene blue agar (Difco, Becton Dickinson) for E. coli selection and identification.

(iv) Histologic evaluation. Samples of uterus, placenta, fetal brain, and lung were fixed in 3% formalin and embedded in paraffin. Paraffin sections (5 μm thick) were stained with standard hematoxylin and eosin techniques. Inflammation was evaluated in a blinded fashion by an investigator using a four-level semiquantitative scoring system based on signs of congestion and edema, necrosis, and inflammatory infiltrate: –, no sign of inflammation; ±, some signs of inflammation; +, moderate signs of inflammation; ++, marked signs of inflammation.

Statistical analysis. Bacterial counts from blood cultures were subjected to one-way analysis of variance according to the different groups, followed by a Scheffe’s test for intergroup comparisons (Statview; Abacus Concepts, Berkeley, Calif.). Percentages were compared by a chi-square test with Yates correction. A P of <0.05 was considered significant.

RESULTS

Choice of experimental conditions. A pilot study involving 20 animals was performed first to determine the experimental conditions providing the most efficient model ensuring a high rate of infection and high fetal survival at the beginning of treatment. Various bacterial inocula (10^3, 10^6, and 10^8 CFU/ml) and various infection periods (12, 24, 36, and 48 h) were tested. The best compromise was an inoculum of 10^6 CFU and a 12-h period, providing 100% maternal positive blood cultures without any maternal death and an 87% fetal survival rate with positive blood cultures in 76% of infected fetuses. A lower initial inoculum gave a lower rate of infected fetuses, whereas a higher inoculum caused early death of the animals. Periods longer than 12 h were associated with an increased maternal and fetal death rate, preventing pertinent evaluation of treatment.

Antibiotic effects on E. coli infection. (i) Survival. At the beginning of antibiotic treatment, 12 h after inoculation of 10^6 CFU E. coli, the survival rate was 100% (5 of 5) and 87% (48 of 55) for control mothers and fetuses, respectively. All mothers without treatment died within the next 24 h, whereas 100% of those treated survived throughout the follow-up period (72 h after the beginning of treatment). Table 1 shows the different fetal survival rates depending on the treatment period and the therapeutic regimen administered. Fetal death was 100% for untreated fetuses, within the second day of infection. The best survival rate was observed with two-drug therapy (χ^2, P < 0.05).

(ii) Bacteriology. Maternal bacteremia at the beginning of treatment showed a mean level of 3.7 ± 0.5 log_{10} CFU/ml (Fig. 1) (data are presented as means ± standard deviations). Maternal blood cultures were sterile after 48 and 72 h of treatment by one- or two-drug therapy, while the bacterial count reached a value of 8.8 ± 0.5 log_{10} CFU/ml in untreated mothers.

Figure 1 also indicates the level of fetal bacteremia in treated and control groups. Twelve hours after inoculation, 4.5 ± 0.7 log_{10} CFU/ml were quantified in fetal blood. Bacteremia increased further, reaching a mean level of 8.8 ± 0.1 log CFU/ml 36 h after inoculation for dead fetuses in the untreated group. No treated fetuses had sterile blood cultures at any time during follow-up. In fact, fetal bacteremia increased by 1 log CFU/ml after 24 h of treatment with ceftriaxone alone,
and continued treatment only allowed the bacterial inoculum to be maintained at this level. Levels of $5.7 \pm 0.6$, $5.2 \pm 0.4$, and $6.2 \pm 0.4$ log CFU/ml were quantified in blood after 24, 48, or 72 h of treatment, respectively. Similarly, the association of gentamicin with ceftriaxone had only a bacteriostatic effect, maintaining fetal bacteremia at around 4 log CFU/ml. Levels of $4.4 \pm 0.9$, $3.9 \pm 1.0$, and $5.2 \pm 0.9$ log CFU/ml were quantified in blood after 24, 48, or 72 h of treatment, respectively.

After 72 h of treatment by ceftriaxone or ceftriaxone plus gentamicin, respectively, 32 of 43 (75%) and 18 of 24 (75%) of placental cultures remained positive.

(iii) Pharmacokinetic parameters. Figure 2 shows the fetal and maternal antibiotic serum concentrations measured after 24, 48, or 72 h of treatment. Ceftriaxone values were usually more than a thousand times higher than the MIC for E. coli in fetuses, suggesting that the concentrations observed were efficient throughout the experimental period. Similarly, the trough concentration of gentamicin observed in fetuses (from 0.3 to 2 mg/liter) indicated that therapeutic concentrations were reached at peak time in treated animals. The progressively higher residual values for gentamicin in fetuses was indicative of an accumulative trend.

**Histologic inflammation.** Table 2 summarizes the results of semiquantitative analysis of inflammation in the uterus, pla-
Ceftriaxone/H11001/chorioamnionitis, with congested villi, neutrophil inflammation was similar in untreated mothers and bacterial challenge. With simulation of human pharmacokinetics, treatment was started 12 h after bacterial inoculation.

Even with two-drug therapy applied for 24 or 72 h, in infected animals treated with the combination of ceftriaxone and gentamicin, marked inflammation in most cases (lung features similar to those observed in the premature infant) (8). In lung of infected fetuses, in contrast to those observed in the premature infant (8), an adequate disease duration and yield (a sufficient number of infected and surviving animals) to allow assessment of treatment effects; (iv) a good reproducibility; (v) a fetal size compatible with various types of tissue sampling; and (vi) a simple experimental technique not conducive to iatrogenic pathologies. E. coli was chosen because the incidence of maternofetal infections involving this microbe appears to be increasing today (25), whereas cases of early group B streptococcal infections are decreasing now that intrapartum prophylaxis has become the general practice (33). Antibiotic therapy was administered in conditions providing the best chance of success, i.e., with bactericidal drugs and serum concentrations higher than the MIC of the microbe. In fact, the association of the two antibiotics used in this study is known to be synergistic and bactericidal in vitro (16). The choice of a broad-spectrum cephalosporin (representing first-line therapy today) was based on the current increase in penicillinase-secreting E. coli strains (29). Ceftriaxone was preferred to cefotaxime for practical reasons, as its binding to rabbit serum proteins is similar to that in humans (11, 18).

Another feature of this model is the effective control of pharmacokinetics in the gestating rabbit based on simulation of human pharmacokinetics for the antibiotics tested, with measurement of the plasma concentrations actually obtained in rabbit mothers and fetuses. This is an important point, as the pharmacokinetics of a product can differ from one animal species to another (e.g., a shorter elimination half-life in a small animal than in humans), and the failure of antibiotic therapy cannot be interpreted in a relevant manner without serum or tissue assays (30). Finally, precise diagnostic practices were used, i.e., quantitative blood cultures, careful avoidance of the carry-over effect by addition of cephalosporin inhibitors in culture media (10).

The most striking result of this study was the contrast between the highly beneficial effect of antibiotic treatment for mothers and the rather slight benefit for fetuses. Without treatment, both mothers and fetuses died around 36 h after the onset of infection. For treated mothers, both survival and blood culture sterilization improved from 0 to 100%. Conversely, fetal survival improved only slightly, while fetal bacteremia remained at a level of around 4 log CFU/ml. These results are in agreement with those of Gibbs et al. (12), who

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a Scored histologic results observed during a rabbit maternofetal E. coli infection model. Microscopic examination of the placenta and fetal lung and brain obtained by cesarean section were performed at various times for treated and untreated animals (n = 8 fetuses in each group). Histology scores: –, no inflammation, normal tissue; ±, slight inflammation with edema and congestion alone, no polymuclear infiltrate; +, moderate inflammation with edema, congestion, and only a few neutrophils; + +, marked inflammation (edema, congestion, presence of an inflammatory infiltrate [mainly neutrophils], and necrosis).

b Uninfected animals.

c Infected and untreated animals 12 and 36 h after bacterial inoculation.

d Infected animals treated with the combination of ceftriaxone and gentamicin, with simulation of human pharmacokinetics. treatment was started 12 h after bacterial challenge.

NA, not available because of 100% fetal death.

DISCUSSION

Despite optimized maternal antibiotic therapy, sterilization of infected fetuses could not be achieved in our experimental model. However, efficient treatment is needed to avoid the complications involved in extremely premature births and to limit the deleterious effects of chorioamnionitis. This type of infection is particularly dangerous for the viability of the fetus and can cause severe sequelae (notably pulmonary and neurological) in the infant at birth (1). A relation has been found between infection and periventricular leukomalacia (a neurological disease in the premature infant) (40), and it appears that the inflammatory environment during this infection is detrimental to the infant’s neurological (39; P. J. Duggan, E. F. Maalouf, T. L. Watts, M. H. Sullivan, S. J. Counsell, J. Allsop, L. Al Nakib, M. A. Rutherford, M. Battin, I. Roberts, and A. D. Edwards, Letter, Lancet 358:1699-1700, 2001) and pulmonary (38) state.

It is also highly debatable whether the alternative of treating this infection by antibiotics administered intrapartum or postpartum is valid. The critical question is whether an infected fetus can be treated in utero. There is no consensus today concerning therapeutic management of chorioamnionitis and the efficacy of prenatal antibiotic therapy (17, 28). Too few comparative clinical trials have been conducted, and empirical practice suggests that neonatal treatment is preferable to fetal treatment (21). Nevertheless, intrapartum treatment, if consistently effective, could be of considerable interest in facilitating neonatal care management. Thus, further investigations are needed, as no reliable recommendations are currently available concerning the most appropriate antimicrobial regimen for an effective cure of intra-amniotic infections.

The present study evaluated the effects of optimized maternal antibiotic therapy in a rabbit maternofetal-infection model offering the following advantages: (i) a fetoplacental unit and hemochorial placentation similar to those of humans; (ii) a natural history of the disease comparable to that observed in humans, notably with neurologic and pulmonary lesions similar to those observed in the premature infant (8); (iii) an adequate disease duration and yield (a sufficient number of infected and surviving animals) to allow assessment of treatment effects; (iv) a good reproducibility; (v) a fetal size compatible with various types of tissue sampling; and (vi) a simple experimental technique not conducive to iatrogenic pathologies. E. coli was chosen because the incidence of maternofetal infections involving this microbe appears to be increasing today (25), whereas cases of early group B streptococcal infections are decreasing now that intrapartum prophylaxis has become the general practice (33). Antibiotic therapy was administered in conditions providing the best chance of success, i.e., with bactericidal drugs and serum concentrations higher than the MIC of the microbe. In fact, the association of the two antibiotics used in this study is known to be synergistic and bactericidal in vitro (16). The choice of a broad-spectrum cephalosporin (representing first-line therapy today) was based on the current increase in penicillinase-secreting E. coli strains (29). Ceftriaxone was preferred to cefotaxime for practical reasons, as its binding to rabbit serum proteins is similar to that in humans (11, 18).

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found that administration of therapeutic antibiotic doses to mothers did not consistently eradicate bacteria from the fetus. The reasons for the failure of antibiotic therapy in this study are not apparent. The transition from fetal state to existence as a newborn corresponds to an abrupt change of environment that is likely to modify response to antibiotics in an infected individual. Changes in blood oxygen supply, resulting from the very sudden increase in blood partial oxygen pressure at birth, could facilitate the action of aminoglycosides, which are known to be sensitive to oxygen. Acidosis and hypoxia within the center of infection could also be conducive to a certain tolerance for beta-lactams and limit the effect of aminoglycosides (6, 37). The uterine environment may also play a role because of anatomic contiguity with the infected fetus. Despite the improvement in maternal survival (negative blood cultures), indicative of a certain efficacy of the treatment for maternal infection, persistent uterine and placental inflammation was observed under antibiotic therapy, as reported elsewhere (12). It is conceivable that placental substances capable of modifying response to antibiotic effects exist, although there is currently no objective evidence for this. The immunological immaturity of the fetus is another possible factor, as altered immune response could limit the efficacy of antibiotic therapy. Some authors have suggested poor tissue penetration of antibiotics (12). In our study, blood concentrations of antibiotics in mothers and fetuses were checked throughout treatment and showed measurements well above the MIC of the microbe concerned. However, these concentrations, though known to be efficient in the usual conditions of antibiotic action in the immunocompetent adult (as indicated by the sterilization of an immunocompetent adult (as indicated by the sterilization of maternal blood cultures in this study), may have been inadequate for fetuses. This point requires further investigation.

In any event, this study shows that fetal infection, though not eradicated, was still not strictly insensitive to treatment. Fetal survival was improved, and bacteriologic quantitative evaluation indicated a bacteriostatic effect in vivo in treated animals, in contrast with continued manifest bacterial growth in control animals. Moreover, a decrease of inflammation was observed in some fetal tissues (lung) under treatment. Although inadequate, these results are promising and favorable to an approach based on maternal antibiotic therapy. They encourage continued research to determine the key factors for successful treatment of maternalfetal infection in utero.

In conclusion, this study indicates the difficulty of obtaining a fetal cure with maternal antibiotic therapy. Further investigations are needed to specify the features of an efficacious antibacterial therapy corresponding to the requirements of this severe infection. The exploitation of the experimental model used here should help elucidate the pathophysiology of maternalfetal infection and perhaps optimize the treatment.

REFERENCES