Characteristics of Murine Model of Genital Infection with *Chlamydia trachomatis* and Effects of Therapy with Tetracyclines, Amoxicillin-Clavulanic Acid, or Azithromycin

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Received 2 December 1993/Returned for modification 27 May 1994/Accepted 30 June 1994

Following intravaginal inoculation of progesterone-treated outbred mice with *Chlamydia trachomatis* MoPn, 4 to 6 log,

inclusion-forming units were recovered in vaginal swabs for 21 days but all animals were culture negative after 28 days. Serum antibody titers were elevated and remained high for at least 70 days. Between 28 and 70 days, upper tract infection (inflammation and distension of the uterine horns, occlusion of oviducts with inflammatory exudate, pyosalpinx, and hydrosalpinx) was seen in >80% of the animals. Mice were dosed orally, commencing at 7 days after infection, with minocycline, doxycycline, or amoxicillin-clavulanate. Further groups received azithromycin either as a single high dose or as lower once-daily doses. In addition, minocycline and amoxicillin-clavulanate were administered at 24 h after infection, and this early treatment prevented elevation of antibody titers whereas delayed therapy did not. Vaginal swabs from mice in all treatment regimens were culture negative except for 25% of mice receiving either early amoxicillin-clavulanate or low-dose azithromycin, which yielded low numbers (20 to 70 inclusion-forming units) of chlamydiae. Numbers of fertile mice in the early treatment regimens and their litter sizes were similar to those of noninfected controls, although 25% of amoxicillin-clavulanate-treated mice had unilateral hydrosalpinges. In comparison, 88% of untreated mice developed hydrosalpinges and only 25% conceived. Delayed dosing did not affect the outcome of amoxicillin-clavulanate therapy but did diminish the protective efficacy of minocycline such that 50% of early RH3 mice had either unilateral hydrosalpinges or ovarian abscessed. Doxycycline and azithromycin were highly effective in restoring fertility. This model makes possible the study of both short- and long-term outcomes of chlamydial infection.

*Chlamydia trachomatis* is a major cause of the world's most common sexually transmitted diseases, with an estimated 50 million new cases being reported annually (7). This organism is responsible for a variety of genital syndromes in men and women (15). Experimental animal models have been of significant value in studying the pathogenesis of chlamydial infection, particularly the immunopathological sequelae of untreated asymptomatic infection in females—salpingitis, ectopic pregnancy, and tubal infertility. Nonhuman primate models resemble the human disease most closely (19), but these animals are costly and in restricted supply. Guinea pigs, rabbits, and cats have also been used (11, 20, 21). For the evaluation of chemotherapeutic agents, the mouse is the most convenient and economical species.

Nevertheless, establishment of infection in mice with human serovars of *C. trachomatis* requires hormonal manipulation, usually of inbred animals, and surgical intervention to place the organisms directly into the upper genital tract via the uterus or ovarian bursa. This procedure is time-consuming but results in salpingitis, altered tubal function, and impaired fertility (29, 30), and the model has been used successfully to evaluate new antimicrobial agents (33). By using the simpler intravaginal route of inoculation, infection can be induced in mice, but uterine horn involvement is serovar dependent (10). Development of salpingitis from this route has been reported to be irregular (31), and the model would therefore be unsuitable for studying the long-term effects of chemotherapy on fertility. The mouse pneumonitis biovar of *C. trachomatis*, MoPn, is genetically and antigenically distinct from human bi variants (32) but has the advantage that it is a natural mouse pathogen, particularly of the respiratory tract. To our knowledge, the use of this strain in experimental genital tract chemotheraphy has been restricted to two studies involving intrabursal inoculation of mice to induce hydrosalpinx and infertility (5, 26) and a third in which *C. trachomatis* MoPn was introduced intravaginally to progesterone-treated mice. In the last study, therapy experiments were terminated after 12 days and long-term sequelae were not described (3).

We report here, for the first time, the development of an upper genital tract infection in progesterone-treated outbred mice caused by intravaginal inoculation with *C. trachomatis* MoPn, in which salpingitis, hydrosalpinx formation, and loss of fertility were observed. We have used the model to assess the effects of minocycline, doxycycline, amoxicillin-clavulanic acid, and azithromycin on chlamydial shedding and preservation of fertility.

**MATERIALS AND METHODS**

**Organism.** *C. trachomatis* MoPn (ATCC VR-123) was grown in cycloheximide-treated McCoy cells by conventional methods. The growth medium was minimal essential medium supplemented with 10% fetal calf serum, 1% nonessential amino acids, 2 mM l-glutamine, 1% vitamins, and 0.5% glucose. Cell lysates containing approximately $5 \times 10^8$ inclusion-forming units (IFU) per ml were prepared and stored at −70°C.

**Susceptibility tests.** Amoxicillin trihydrate and potassium clavulanate were laboratory reference powders (SmithKline

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Beecham Pharmaceuticals, Worthing, United Kingdom [UK]. Minocycline and doxycycline hydrochloride were purchased from Sigma Chemical Co. Ltd., Poole, UK, and azithromycin was a commercial preparation (Pfizer, Sandwich, UK). Solutions of the pure free acids were prepared in distilled water for minocycline, doxycycline, and clavulanic acid and in phosphate-buffered saline (PBS; pH 8) for amoxicillin. Azithromycin was dissolved in 5% (vol/vol) ethanol. Minocycline-clavulanate was tested in a 2:1 ratio. McCoy cell monolayers on coverslips in 24-well plates were inoculated with approximately 900 IFU of C. trachomatis MoPn per well, and infection was enhanced by centrifugation at 1,400 × g for 1 h. After incubation for a further 1 h, the inoculant was withdrawn and replaced with chlamydial growth medium containing doubling dilutions of the antibiotics and 1 μg of cycloheximide per ml. The plates were incubated for 48 h, and the coverslips were stained with Giemsa stain. The MIC was the lowest concentration preventing normal inclusion development as determined under examination by phase contrast microscopy. One drug-free passage was performed to obtain an MBC.

Animals. Female MF1 mice (Harlan OLAC), weighing 18 to 22 g, were used throughout and were given access to food and water ad libitum. For fertility tests, proven stud males of the same strain were used.

Drug pharmacokinetics. Nonfasted, noninfected mice were dosed orally by gavage with minocycline, doxycycline, amoxicillin-clavulanate, or azithromycin. For administration to mice, azithromycin was suspended in gum acacia. At intervals, groups of five mice per treatment were killed by CO2 asphyxiation, and blood was collected by cardiac puncture. Serum was obtained by centrifugation of the blood samples at 15,000 × g. Genital tract tissues were removed and homogenized in PBS by Griffin’s tubes. Concentrations of antibiotics in serum and tissues were determined by microbiological assay, with Bacillus cereus BRL1239 for minocycline and doxycycline and Staphylococcus saprophyticus NCTC8340 for amoxicillin and azithromycin, and the Klebsiella pneumoniae NCTC11228 β-lactamase inhibition assay was used for clavulanic acid. Standards were prepared in pooled mouse serum and genital tract tissue homogenate. The doses selected for efficacy studies were those which produced, in mouse serum, peak concentrations similar to those obtained in humans following standard oral dosage of these agents. In the case of azithromycin, concentrations in genital tract tissue were used as a guide to dose selection.

Inoculation. Animals received 2.5 mg of progesterone subcutaneously (Depo-Provera; Upjohn, Crawley, UK), and a second dose was given 7 days later. One hour after the second dose, mice were anesthetized by intraperitoneal injection with 60 mg of pentobarbitone per kg of body weight. The vaginal opening was exposed, and 20 μl of undiluted chlamydial suspension was added. Control animals received sterile growth medium.

Drug treatment. The tetracyclines were given twice daily, minocycline at 5 mg/kg and doxycycline at 10 mg/kg. Amoxicillin-clavulanate (20/10 mg/kg) was given three times daily, to compensate for the shorter half-lives of the β-lactams in the mouse. Azithromycin was given either as a single oral dose of 80 mg/kg or as a single dose of 40 mg/kg followed by three once-daily doses of 20 mg/kg. All other treatments were administered orally for 10 days, to mimic the regimen often recommended for treatment in humans (28). Minocycline and amoxicillin-clavulanate treatment commenced at either 24 h or 7 days after inoculation, and doxycycline and azithromycin treatment commenced at 7 days only. Control infected mice received saline. The effects of therapy were assessed by measuring serum antibody titers and vaginal shedding of chlamydial one day after cessation of therapy in eight mice per treatment, in order to obtain an immediate determination of the effect on chlamydial load. The longer-term outcome of therapy was assessed by fertility tests conducted on the remaining eight animals between 4 and 12 weeks after infection. Genital tissues from infected mice, treated and untreated, were processed for histopathological examination at 28 days after infection.

Serum antibody titers. Blood was collected by cardiac puncture and centrifuged to obtain serum. Twofold dilutions were prepared in PBS, pH 7.2, and total antibody levels were measured with an indirect immunofluorescence kit developed for clinical use (Chlamydia Spot-IF; API-BioMerieux, Charbonnieres-les-Bains, France), with which fluorescein isothiocyanate-conjugated anti-mouse globulin raised in rabbits (Se-rotec, Oxford, UK) was used as a substitute for anti-human globulin. Results were expressed as the reciprocal of the lowest dilution giving a positive result.

Isolation of chlamydiae. Dry nasopharyngeal swabs were inserted into the vagina. The canal and exocervix were vigorously scraped, and swabbed material was expressed into 2× sucrose phosphate transport medium and frozen immediately at −70°C. All specimens were processed on the same day. Thawed undiluted samples were plated in 0.5-m1 volumes onto McCoy cells on coverslips (13-mm diameter) in 24-well plates, and infection was enhanced by centrifugation. Coverslips were stained with Giemsa stain after 48 h of incubation, and results were expressed as log10 IFU per milliliter of sample.

Fertility tests. At 4 weeks after infection, the remaining animals were separated and housed individually with stud males in a 1:1 ratio, for 10 days (33). Litter sizes were recorded, and postmortem examinations were carried out on the mothers. Animals not conceiving during the first mating period were rehoused with the males 4 weeks later. All mice were terminated at 12 weeks after infection.

Histopathology. Entire genital tracts were fixed in formalin and processed to wax blocks. Sections were hematoxylin and eosin stained for histological examination.

Statistical analysis. Data were analyzed by t test with the Wilk-Shapiro test for normal distribution. P values of 0.05 or less were considered significant.

RESULTS

Susceptibility in vitro. C. trachomatis MoPn was highly susceptible to minocycline and doxycycline, which were bactericidal at 0.008 and 0.031 μg/ml, respectively (Table 1). Amoxicillin and clavulanic acid were inhibitory at 0.25 and 0.5 μg/ml, respectively, and the activity of the combination reflected an additive interaction. At concentrations above the MICs of the β-lactams, abnormal inclusions which were either empty or contained very large reticulate bodies were seen. On removal of the β-lactam agents, normal inclusions were observed over the entire test range. Azithromycin had inhibitory activity.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MIC (μg/ml)</th>
<th>MBC (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>0.25</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Clavulanic acid</td>
<td>0.5</td>
<td>&gt;16</td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid</td>
<td>0.12/0.062</td>
<td>&gt;32/16</td>
</tr>
<tr>
<td>Minocycline</td>
<td>0.004</td>
<td>0.008</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>0.008</td>
<td>0.031</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>0.25</td>
<td>0.25</td>
</tr>
</tbody>
</table>
TABLE 2. Comparative pharmacokinetic data for minocycline, doxycycline, amoxicillin-clavulanic acid, and azithromycin in mice and humans

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mice</th>
<th>Result for*:</th>
<th>Humans</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dose (mg/kg)</td>
<td>Concen (μg/ml; mean ± SE)</td>
<td>T_{1/2} (h)</td>
</tr>
<tr>
<td>Minocycline</td>
<td>5</td>
<td>2.4 ± 0.4*</td>
<td>0.9</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>10</td>
<td>2.8 ± 1.5*</td>
<td>1.7</td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid</td>
<td>20</td>
<td>4.3 ± 1.8*</td>
<td>0.3</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>10</td>
<td>2.9 ± 1.0*</td>
<td>0.22</td>
</tr>
<tr>
<td>Clavulanic acid</td>
<td>80</td>
<td>16.8 ± 4.7*</td>
<td>20.2</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>40</td>
<td>7.5 ± 2.6*</td>
<td>32.0</td>
</tr>
</tbody>
</table>

* Abbreviations: T_{1/2}; half-life at β phase; C_{max}; peak concentration of drug in serum.
* Measured in serum at 45 min.
* Data are from reference 22.
* Measured in serum at 15 min.
* Measured in serum at 30 min.
/ Data are from reference 27.
* Measured in genital tract tissue at 480 min.
* In micrograms per gram.
/ Measured in genital tract tissue at 240 min.
* Data are from reference 9.

similar to those of amoxicillin and amoxicillin-clavulanic acid but was also bactericidal at this concentration.

Pharmacokinetics. Forty-five minutes after an oral dose of 5 mg of minocycline per kg to mice, a mean peak concentration of 2.43 ± 0.44 (mean ± standard error) µg/ml was measured in serum (Table 2). Levels of 0.30 ± 0.12 µg/ml were still present at 3 h, resulting in a prolonged half-life of 0.9 h. Similar peak concentrations of doxycycline after a 10-mg/kg dose were measured earlier at 15 min, but the half-life was longer than that of minocycline, at 1.7 h (Table 2). Concentrations of amoxicillin and clavulanic acid were 4.33 ± 1.79 and 2.91 ± 1.01 µg/ml, respectively, at 30 min after a dose of 20/10 mg/kg, but these compounds were eliminated more rapidly than the tetracyclines, with half-lives of 0.3 h for amoxicillin and 0.22 h for clavulanic acid. After administration of azithromycin at 40 and 80 mg/kg, peak concentrations in genital tract tissue homogenates were 7.5 ± 2.6 and 16.8 ± 4.7 µg/g, respectively. This agent was still detectable in tissues at 48 h, and computed half-lives were between 20 and 30 h. The concentrations in serum reported here were of the same order as the peak concentrations in serum reported in humans after administration of 150 mg of minocycline, 200 mg of doxycycline, or 500/250 mg of amoxicillin-clavulanic acid, but the half-lives in mouse serum were significantly shorter than those in humans (Table 2). Concentrations of azithromycin in mouse genital tract tissue after a dose of 40 mg/kg were similar to those measured in human tissues following a 500-mg dose.

Course of infection. In these studies, progesterone-treated

FIG. 1. Recovery of C. trachomatis MoPn in vaginal swabs from mice after intravaginal inoculation (open circles, counts from individual animals; data are means ± standard errors [bars]) and development of hydrosalpinges (closed circles).
mice received approximately 1,000 IFU of *C. trachomatis* MoPn intravaginally. Seven days later, a mean count of 5.55 ± 0.22 log_{10} IFU/ml was recovered from vaginal swabs from untreated animals (Fig. 1). From 14 days onwards, a gradual decline in chlamydial shedding occurred, and six of eight animals examined at 28 days were culture negative. The disappearance of viable organisms between 21 and 28 days was, however, paralleled by development of an upper genital tract infection, characterized initially by distension and inflammation of the uterine horns, pyosalpinx, and hyperproduction of mucous exudate at around 21 days. Histological examination of oviducts 28 days after infection revealed swelling of the subepithelial connective tissue in comparison with uninfected oviducts (Fig. 2A and B). Inflammatory exudate partially occluded the lumen. Numerous polymorphonuclear leukocytes were evident, but no marked destruction of the columnar epithelium was seen at this stage. Finally, development of hydrosalpinges occurred in more than 80% of the mice after 28 days and was still evident at 10 weeks (Fig. 3).

**Early effects of therapy.** To determine the short-term efficacy of therapy, vaginal swabs and serum samples were taken from animals 1 day after cessation of a 10-day course of oral treatment with minocycline, doxycycline, or amoxicillin-clavulanate. This was designed to assess the reduction in chlamydial load in response to treatment. Although the regimens of azithromycin were shorter, these treated mice were sampled at the same time as animals in the other groups. All animals treated with minocycline and doxycycline were culture negative (Table 3). After early treatment with amoxicillin-clavulanate and the lower-dose regimen of azithromycin, six of eight mice in each group were culture negative, with counts close to the detectable limit (one inclusion per coverslip) being recovered from the remaining animals. Interestingly, no chlamydiae were recovered after delayed amoxicillin-clavulanate therapy, but this difference was not considered significant. Likewise, all mice receiving the high dose of azithromycin were culture negative (Table 3). Elevated antichlamydial antibody levels were measured in serum of untreated mice from 3 to 4 days after inoculation, and these remained high for at least 12 weeks (data not shown). Elevation of titers was substantially prevented when treatment commenced at 24 h after infection, but not at 7 days (Table 4). At the microscopic level, oviducts from mice treated at 7 days with either minocycline or amoxicillin-clavulanate were substantially devoid of signs of infection at 28 days and were similar in appearance to tissues from noninfected mice.

**Long-term effects of therapy.** To assess the outcome on the long-term consequences of this infection, fertility tests were conducted between 4 and 12 weeks after inoculation. Noninfected females produced total litters of 84, 86, and 96 in the three studies reported (Fig. 4A and B and 5) with an average

![Fig. 2. Sections of mouse oviducts, noninfected (A) and 28 days after intravaginal inoculation with *C. trachomatis* MoPn (B) (hematoxylin and eosin stain; magnification, ×50).](image)

**FIG. 3. Female mouse genital tract 70 days after intravaginal inoculation with *C. trachomatis* MoPn, showing bilateral hydrosalpinges.**

![Fig. 3](image)

**TABLE 3. Effects of therapy with minocycline, doxycycline, amoxicillin-clavulanic acid, or azithromycin on the recovery of *C. trachomatis* MoPn from vaginal swabs from infected mice.**

<table>
<thead>
<tr>
<th>Treatment (dose [mg/kg])</th>
<th>Concen (log_{10} IFU/ml [mean ± SE]) in vaginal swabs when therapy started at:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h^a</td>
</tr>
<tr>
<td>None</td>
<td>5.01 ± 0.51</td>
</tr>
<tr>
<td>Minocycline (5)</td>
<td>&lt;0.5^c</td>
</tr>
<tr>
<td>Doxycycline (10)</td>
<td>ND^d</td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid (20/10)</td>
<td>0.75 ± 0.50</td>
</tr>
<tr>
<td>Azithromycin (80)</td>
<td>ND</td>
</tr>
<tr>
<td>Azithromycin (40 or 20)</td>
<td>ND</td>
</tr>
</tbody>
</table>

^a Samples taken 11 days after infection.
^b Samples taken 18 days after infection.
^c Limit of detection.
^d ND, not determined.
TABLE 4. Effects of therapy with minocycline or amoxicillin-clavulanic acid on antibody titers in mice infected with C. trachomatis MoPn

<table>
<thead>
<tr>
<th>Infection status and treatment (dose [mg/kg])</th>
<th>Reciprocal serum antibody titers (mean ± SE)* 1 day after cessation of therapy started at:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
</tr>
<tr>
<td>Infected</td>
<td>7 days</td>
</tr>
<tr>
<td>None</td>
<td></td>
</tr>
<tr>
<td>Minocycline (5)</td>
<td>128 ± 7</td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid (20/10)</td>
<td>24 ± 8</td>
</tr>
<tr>
<td>Noninfected</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>32</td>
</tr>
<tr>
<td>* n = 4.</td>
<td></td>
</tr>
</tbody>
</table>

of 10.5 to 12.0 pups per female (Table 5). In contrast, the litters produced by infected untreated mice were significantly smaller (31, 18, and 11), reducing the average number of pups per female to between one and four. On postmortem, seven of eight (88%) infected mice in each study had developed hydrosalpinges. These were bilateral except for one (Fig. 4A) and two (Fig. 4B and 5) animals which had a single hydrosalpinx, permitting normal conception on the contralateral side, albeit with a reduced number of implantations. Mice which had received minocycline or amoxicillin-clavulanate at 24 h after infection produced identical total litter sizes (Fig. 4A and B) which were actually slightly but not significantly larger than those in the noninfected controls (Table 5). Two amoxicillin-clavulanate-treated animals which gave birth to six and eight pups were found to have a unilateral hydrosalpinx. Delayed therapy did not have a detrimental effect on the efficacy of amoxicillin-clavulanic acid in preserving fertility, and the incidence of hydrosalpinx formation was not increased (Fig. 4B). Minocycline-treated mice were free of gross pathological abnormalities following early institution of therapy, but when treatment was delayed to 7 days the total litter was smaller than that produced by noninfected or amoxicillin-clavulanate-treated mice (Fig. 4B and Table 5). Two of four animals had developed a unilateral hydrosalpinx. The other two mice in this group which failed to conceive did not have hydrosalpinges but had enlarged ovaries with pustular lesions. In contrast, a single mouse on the doxycycline regimen had a unilateral hydrosalpinx, and the group produced a normal litter size (Fig. 5). Both azithromycin regimens were highly effective in preventing hydrosalpinx formation (Fig. 5).

FIG. 5. Effects of oral therapy with doxycycline or azithromycin on the development of hydrosalpinges in mice previously infected with C. trachomatis MoPn. Therapy commenced at 7 days after infection. The bars indicate percentages of mice with bilateral (plain) or unilateral (shaded) hydrosalpinges. The figures in parentheses indicate total litter size produced in each treatment group (n = 8).

FIG. 4. Effects of oral therapy with minocycline or amoxicillin-clavulanic acid on the development of hydrosalpinges in mice previously infected with C. trachomatis MoPn. Therapy commenced at 24 h (A) or 7 days (B) after infection. The bars indicate percentages of mice with bilateral (plain) or unilateral (shaded) hydrosalpinges. The figures in parentheses indicate total litter size produced in each treatment group (n = 8).

DISCUSSION

Intravaginal inoculation of progesterone-treated MF1 mice produced a biphasic infection. The early stage was characterized by shedding of chlamydiae in vaginal secretions, with numbers gradually declining over a 4-week period. This was accompanied by elevated antichlamydial serum antibody titers, uterine horn inflammation, pyosalpinx, and bursal distension. After this time, no organisms could be detected. The later stage began from 3 to 4 weeks after infection, when hydrosalpinx formation was noted in an increasing proportion of the mouse. By 10 weeks, the majority had bilateral hydrosalpinges and were infertile as a consequence. The course of events in this model was similar to that seen after inoculation with C. trachomatis MoPn or human serovars by the technically more demanding intruterine or intrabursal routes (25, 30). Thus, many of the sequelae of chlamydial infection in women have been reproduced relatively simply in this mouse model. Moreover, these findings represent a significant contrast to other reports on the effect of intravaginal inoculation with C. trachomatis MoPn, in which the infection was described as mild and restricted only to the lower genital tract (1, 3).
Although the mouse pneumonia biovar is distinct from human serovars of C. trachomatis, it was found to share their antibiotic susceptibility characteristics. There is a paucity of information on antimicrobial activity against C. trachomatis MoPn in vitro. Our studies showed that this organism was highly susceptible to minocycline and doxycycline, as are human strains (4, 24). Likewise, amoxicillin, clavulanic acid, and amoxicillin-clavulanic acid were inhibitory at low concentrations but, in keeping with other penicillins, failed to exert a complete bactericidal effect in cell culture, as was demonstrated by Bowie with clinical isolates (6). The susceptibility of C. trachomatis MoPn to azithromycin was similar to that of human strains (23).

By commencement of therapy at 24 h, the efficacy of treatment in preventing development of infection was assessed. This was reflected in the suppression of serum antibody response by early but not by late therapy, confirming the observations of others (33). Therapy started at 7 days was a stricter test for the elimination of an established infection. Chlamydial shedding was generally abrogated by all treatments, regardless of the starting time. This was reflected in the outcome of the fertility tests on mice receiving early treatment, in that the unilateral hydrosalpinges in a small percentage of amoxicillin-clavulanate- or azithromycin-treated mice may have been due to the persistence of low numbers of organisms in some animals after cessation of therapy. Delaying therapy did not appear to diminish the activity of amoxicillin-clavulanic acid in fertility tests, but the efficacy of minocycline was unexpectedly reduced. Under these conditions, therefore, doxycycline was more efficacious than minocycline. Landers and colleagues, using the same organism inoculated directly into the upper genital tract, found that the efficacy of tetracycline was greatly reduced when given at 5 days compared with treatment at 2 days (13). Only our results with minocycline appear to confirm this, which may be due to the fact that establishment of infection in the oviducts takes longer following intravaginal inoculation. A single dose of azithromycin was highly effective in preventing chlamydial shedding and therefore in preserving fertility, and this observation is in keeping with clinical experience in chlamydial cervicitis (17).

In these tests, no attempt was made to detect chlamydiae in the later stages, since the organism ceased to be culturable by conventional means after 4 weeks. Techniques such as PCR may provide information additional to that provided by the fertility tests in establishing the long-term effects of therapy, but it is important that the distinction can be made between detection of dead cell fragments and that of latent but viable bacteria.

At clinically achievable concentrations in serum, the activity of amoxicillin-clavulanic acid in these studies was markedly better than might be expected from that seen in cell culture, as we have found in a murine respiratory infection caused by the same organism (2). Amoxicillin has been shown by other researchers to have good activity in this respiratory model (12). Although reservations over the use of penicillins for chlamydial infection because of their incomplete bactericidal effect exist, amoxicillin has proven to be a useful alternative to standard treatment of chlamydial infection in pregnancy (14), in cervicitis (18), and in male urethritis (8). The established broad spectrum of activity of amoxicillin-clavulanic acid (16) means that it has potential in the treatment of polymicrobial genital infections involving β-lactamase-producing bacteria. Thus, the antichlamydial activity reported here may have some significance in situations in which C. trachomatis is implicated.

The infection which was induced following intravaginal inoculation of mice with C. trachomatis MoPn was similar to that produced by direct instillation of organisms into the upper genital tract, and the model offers a simpler but equally informative means of predicting the antichlamydial potential of antimicrobial agents. Moreover, this model may have utility in the study of chlamydial latency and the interaction between this intracellular pathogen and the immune response.

ACKNOWLEDGMENTS

We thank members of Laboratory Animal Sciences for their invaluable assistance with these studies and Sheila Smith and Michael Wilkinson, Analytical Sciences, for provision of the histology data.

REFERENCES


