Observations on the antibiotic treatment of experimentally induced mycoplasmal infections in mice

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Antibiotics were given subcutaneously to female mice, colonized by mycoplasmas in the vagina and by others in the oropharynx. *Mycoplasma pulmonis* was eradicated from the vagina of 36 of 42 immunocompetent TO or BALB/c mice with oxytetracycline, and from 17 of 18 TO mice with lymecycline. *Mycoplasma hominis* was eradicated from the vagina of all 18 immunocompetent BALB/c mice with oxytetracycline or tetracycline. Regarding oropharyngeal organisms, *M. pulmonis* was eradicated from only 20 of 42 TO or BALB/c mice with oxytetracycline and from none of 15 mice with lymecycline. However, *Mycoplasma pneumoniae* was eliminated from the oropharynx of eight of nine BALB/c mice with oxytetracycline. In contrast to the success in eradicating mycoplasmas from the vagina or oropharynx of immunocompetent BALB/c mice with oxytetracycline, no such effect was seen in nude BALB/c mice, indicating the importance of a competent immune system in conjunction with antibiotic treatment for eradication of these organisms.

Materials and methods

Mice and hormonal treatment

Adult female mice, 18–22 g and 6–8 weeks old, of strains TO and BALB/c, and also of the athymic nude strain BALB/c-nu, were used. They were screened for, and deemed to be free of, indigenous mycoplasmas before use.

Progesterone (Depo-Provera; Upjohn, Crawley, UK), 2.5 mg for mice given *M. pulmonis* or *M. pneumoniae*, or oestradiol (Intervet UK, Science Park, Cambridge, UK), 0.5 mg for mice given *Mycoplasma hominis*, was injected in a 0.1 mL volume subcutaneously on four occasions at weekly intervals.

*Mycoplasma medium, mycoplasma inocula and mouse inoculation*

Glucose-containing medium was used for the growth and isolation of *M. pulmonis* and *M. pneumoniae* from murine specimens. Likewise, arginine-containing medium was used for the growth of *M. hominis*. Glucose-containing medium was used for the growth and isolation of *M. pulmonis* and *M. pneumoniae* from murine specimens.

Introduction

Mycoplasmas are bound by a triple-layered membrane and, unlike conventional bacteria, do not have a rigid cell wall. Hence, they are not susceptible to penicillins and other antibiotics that act on this structure. They are, however, susceptible to a variety of broad-spectrum antibiotics, most of which only inhibit their multiplication and do not kill them.1 The tetracyclines have always been in the forefront of the treatment of mycoplasmal infections in humans, but macrolides are also widely used, particularly against infections caused by *Mycoplasma pneumoniae*.

Mice are susceptible naturally to *Mycoplasma pulmonis* and colonization of the oropharynx has been demonstrated experimentally with other mycoplasmas, for example, *M. pneumoniae*.2 In addition, colonization of the genital tract of mice with various mycoplasmas may be induced by prior hormone treatment of these animals.3 We have taken advantage of the mouse models of colonization to assess whether they can be used to determine the effectiveness of antibiotics in vivo and in this context (i) whether different mouse strains and mycoplasmal species behave differently, (ii) whether the response to therapy is different in the genital tract and in the oropharynx and (iii) whether a competent immune system is important in mycoplasmal elimination following antibiotic therapy.

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for *M. hominis*. The components of these media have been described in detail previously.4

Three mycoplasmal species were used. The JB isolate of *M. pulmonis*, of multiple passage *in vitro*, was known to cause pneumonia and arthritis in mice.5 Isolate MY17288 of *M. hominis*, which had received three passes in mycoplasmal broth medium, had been used previously to infect the genital tract of mice.6 Isolate MY12763 of *M. pneumoniae*, which had received five passes in mycoplasmal broth medium, had been used previously to infect both the respiratory and genital tracts of mice.2

Intranasal inoculation of organisms was undertaken only on animals that had been anaesthetized. The mice were injected intraperitoneally with a mixture of one part Hypnorm (fentanyl/fluanisone; Janssen, Grove, UK), two parts water for injection and one part Hypnovel (Midazolam; Roche, Welwyn Garden City, UK). This anaesthetic was administered on the basis of 10 mL/kg bodyweight (0.2 mL per 20 g mouse). During the period of anaesthesia, 50 µL of a suspension of the mycoplasmal organisms were introduced intranasally using a Finnpipette (Labsystems Jencons, Leighton Buzzard, UK) and nalaxone hydrochloride 0.1 mL/kg (Du Pont, Stevenage, UK) was administered intraperitoneally to counteract the effects of the anaesthetic.

Intravaginal inoculation of mycoplasmal organisms was undertaken on unanaesthetized mice. The suspension of organisms in a volume of 50 µL was introduced with a Finnpipette immediately after the second inoculation of hormone.

**Antibiotics and administration**

Oxytetracycline (Engemycin; Mycofarm Ltd, Braintree, UK), oxytetracycline (Occrycetin; Willows Francis Veterinary, Crawley, UK), oxytetracycline (Terramycin; Pfizer, Sandwich, UK) and lymecycline (Tetralysal; Farmitalia, St Albans, UK) were used. Each antibiotic was prepared as a solution in sterile distilled water and given subcutaneously in a volume of 0.1 mL. The dose, frequency and duration of administration are shown in the Table.

**Collection of specimens and estimation of the number of viable mycoplasmas**

A nasopharyngeal swab (MW142; Medical Wire and Equipment Co., Corsham, UK) was inserted into the oropharynx or vagina, rotated to abrade the epithelial cells, and the contents were expressed in 1.8 mL of glucose-containing or arginine-containing mycoplasmal broth medium. Further 10-fold dilutions up to 10⁻⁸ were prepared in mycoplasma medium from the inoculated broth and these cultures were incubated at 37°C. They were observed regularly for about 2 weeks in the case of *M. pulmonis* and *M. hominis* and for about 6 weeks in the case of *M. pneumoniae*. A change in the colour of the medium from red to yellow was seen with growth of *M. pulmonis* or *M. pneumoniae* and from yellow to red with growth of *M. hominis*. The highest dilution to show a change was considered to contain one colour-changing unit (ccu) and this was recorded as the titre of the mycoplasmal culture.

**Results**

**Treatment of immunocompetent TO or BALB/c mice infected genitally with *M. pulmonis***

*M. pulmonis* organisms, although present usually in large numbers in the vagina of TO mice, could not be detected at this site in most of the mice 7–14 days after treatment with oxytetracycline (Engemycin and Terramycin) or lymecycline (Tetralysal) (Table). The organisms persisted in large numbers for at least 14 days in mice that were untreated. In addition, *M. pulmonis* was eliminated from the vagina of eight of nine BALB/c mice by treatment with oxytetracycline (Occrycetin).

**Treatment of immunocompetent BALB/c mice infected genitally with *M. hominis***

Oxytetracycline (Occrycetin), in the same dose and regime as used to treat *M. pulmonis*-infected mice, had eradicated *M. hominis* from the vagina of all of eight BALB/c mice when they were examined 10 days after the course of antibiotic, even though the organisms were present originally in large numbers (titre range 10⁶–10⁸ ccu; geometric mean titre 1.9 × 10⁵ ccu). In contrast, the organisms persisted in large numbers (titre range 10⁵–10⁷) in the vagina of eight of 10 mice that had not been treated. Furthermore, tetracycline hydrochloride (Achromycin) eliminated *M. hominis* from the vagina of all of 10 BALB/c mice that were infected.

**Treatment of immunocompetent TO or BALB/c mice infected oropharyngeally with *M. pulmonis***

As shown in the Table, whereas oxytetracycline or lymecycline eliminated *M. pulmonis* from the vagina of 53 (88%) of 60 TO or BALB/c mice, they did so from the oropharynx of only 22 (37%) of 59 TO or BALB/c mice, despite there usually being fewer organisms in the oropharynx than in the vagina. Occrycetin was most effective, seven of eight BALB/c mice being treated successfully.

**Treatment of immunocompetent BALB/c mice infected oropharyngeally with *M. pneumoniae* or *M. hominis***

Nineteen BALB/c mice were inoculated intranasally with *M. pneumoniae* and the organisms were recovered from...
<table>
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<th>Mouse strain</th>
<th>Antibiotic</th>
<th>Dose daily (mg)</th>
<th>Duration (days)</th>
<th>Days since end of treatment</th>
<th>No. of mice with vaginal organisms</th>
<th>Titre of vaginal organisms (ccu)</th>
<th>No. of mice with throat organisms</th>
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aIn vitro MIC 0.3–0.6 mg/L. GM, geometric mean.
the oropharynx of all of them 14 days later (titre range $10^1$–$10^3$ ccu; geometric mean titre $6.3 \times 10^2$ ccu). Ten of these mice were not treated and *M. pneumoniae* organisms (titre range $10^1$–$10^4$ ccu) were recovered intermittently from six of them for up to 230 days. Eighteen days after intranasal inoculation, the other nine mice were treated with oxytetracycline for 4 days. *M. pneumoniae* was recovered from only one of these mice after 14 days, this animal remaining positive for up to 230 days. The remaining eight mice were negative for a similar period.

Eight mice harboured *M. hominis* organisms in small numbers (titre range $10^1$–$10^3$ ccu) in the oropharynx. Absence of the organisms from this site of three mice 35 days after the administration of oxytetracycline (Occrycetin) was probably unrelated to the treatment, as the organisms had also disappeared by this time from the five untreated mice, suggesting transient existence.

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**Treatment of nude mice infected genitally with *M. pulmonis* or oropharyngeally with *M. pneumoniae***

Oxytetracycline (Occrycetin) eliminated large numbers of *M. pulmonis* organisms from the vagina of eight of nine immunocompetent BALB/c mice (Table), but it failed to do so from the vagina of any of eight nude BALB/c mice.

Eight immunocompetent BALB/c mice, all of which were colonized in the oropharynx with *M. pneumoniae* organisms (titre range $10^1$–$10^6$ ccu; geometric mean titre $1 \times 10^3$ ccu), were treated for 5 days with oxytetracycline. The organisms could not be recovered from the oropharynx of any of the mice 21 and 42 days after the end of treatment. In contrast, the organisms in the oropharynx of eight nude BALB/c mice (titre range $10^1$–$10^6$ ccu; geometric mean titre $2.5 \times 10^3$ ccu) that were treated in the same way persisted for at least 42 days in all of them (titre range $10^1$–$10^4$ ccu).

**Discussion**

The mice were given antibiotics subcutaneously to ensure the accuracy of the dose, which might not have been the case if the antibiotic had been administered in the drinking water. Oxytetracycline and lymecycline were effective in eliminating *M. pulmonis* from the genital tract of most of the immunocompetent mice. Treatment of mice infected genitally with *M. hominis* seemed marginally more successful, as both oxytetracycline and tetracycline eradicated this mycoplasma from all of the mice. Clearly, the model should lend itself to the testing of other doses, other antibiotics or combinations of antibiotics, and other mycoplasmal species.

Although *M. pulmonis* was cleared almost always from the genital tract of immunocompetent TO mice, it could not be eradicated so easily from the throat of these mice even though the concentration of organisms detected at this site was much less than in the genital tract. Elimination of *M. pulmonis* and *M. pneumoniae* from the oropharynx was achieved best in immunocompetent BALB/c mice. It is interesting to note that eradication of *M. pneumoniae* organisms from the human oropharynx has been reported also to be difficult, even though the concentration of antibiotic (a tetracycline) in the respiratory secretions was at least as great as that required to be effective in vitro. The invasion of epithelial cells by mycoplasmas, including *M. pneumoniae*, may provide partial protection.

In contrast to the effectiveness of antibiotics in immunocompetent mice, treatment of nude mice was unable to eliminate *M. pulmonis* from the genital tract or *M. pneumoniae* from the oropharynx. Such persistence is in concert with some of the observations made in the human situation when there is impaired immunity. Thus, failure to eradicate mycoplasmas from infected joints even after iv antibiotic therapy has been seen in hypogammaglobulinaemic patients with a mycoplasma-induced arthritis. Furthermore, *Mycoplasma fermentans* has been detected repeatedly in peripheral blood mononuclear cells of some AIDS patients despite their receiving multiple courses of antibiotics for other intercurrent infections (J. Ainsworth and D. Taylor-Robinson, unpublished data). Support for the notion that successful antibiotic intervention in a mycoplasmal infection depends to a large extent on the ability of the host to mount an adequate immune response also comes from the difficulties experienced in controlling mycoplasmal infection in plants and of eradicating contaminating mycoplasmas from cell cultures, both situations where a functioning immune system does not exist.

**References**


Antibiotic treatment of mycoplasma-infected mice


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