HYPOTHESIS

The case for further treatment studies of uncomplicated genital *Chlamydia trachomatis* infection

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Sex Transm Infect 2006;**82**:340–343. doi: 10.1136/sti.2005.019158

Azithromycin 1 g immediately and doxycycline 100 mg twice daily have good antimicrobial activity against *Chlamydia trachomatis* and treatment studies have demonstrated a >95% microbiological cure at 2–5 weeks, with antimicrobial resistance being rarely reported. Recently an 8% (95%, CI 5% to 11%) failure rate was observed in 289 women, but not in men, who had been sexually inactive after treatment. At high multiplicities of infection (load) in vitro persistence can often be demonstrated to antimicrobials—heterotypic resistance. The subsequently recovered isolates do not possess antimicrobial resistance at low loads. It is known that genital chlamydia load varies in vivo and is probably greater in women than men. In mass treatment trials of trachoma, treatment failure is associated with high chlamydia loads. It is therefore possible that women with high chlamydia loads may be at increased risk of treatment failure. Given the imminent role out of the National Chlamydia Screening Programme and the consequences of persistent chlamydial infection in women this hypothesis urgently merits further investigation.

Until recently, the evidence that there was effective therapy (>95% microbiological cure) for treating uncomplicated *Chlamydia trachomatis* genital tract infection would have been considered to be strong. Azithromycin 1 g immediately and doxycycline 100 mg twice daily for 7 days have been the most rigorously investigated. Comparative studies with follow up periods from 2–5 weeks have demonstrated similar efficacy, with >95% being chlamydia negative on retesting using a nucleic acid amplification technique (NAAT) or culture.[1] Indeed, the concluding remark from the meta-analysis[1] was that comparative studies were no longer necessary. As a result, tests of cure following treatment with either regimen are not recommended.[2] When patients are followed up for longer periods following treatment with either azithromycin or doxycycline, more than 10% will be chlamydia positive on retesting (Scott La Montagne, personal communication).[3 4] This has been considered to be as a result of re-infection, either through exposure to an untreated partner or sexual contact with a new partner. However, there is now emerging evidence, in females treated with azithromycin 1 g immediately and probably doxycycline 100 mg twice daily for 7 days, that this may be the result of both the re-emergence of persistent latent (non detectable) infection as well as re-infection.[3 5 6]

Golden *et al* recently observed in a partner treatment study that 164 (15%) of 1164 women, both asymptomatic and symptomatic, were chlamydia positive when retested 3–20 weeks following azithromycin treatment.[6] Of those completing the study, 78% were originally treated with azithromycin, and 19% were treated with doxycycline, with most others treated with ofloxacin. Comparable levels of treatment failure were observed with both drugs (Matthew Golden, personal communication). Of those retested, 289 had not been sexually active following treatment, of whom 22 (8% (95%, CI 5% to 11%)) were chlamydia positive at follow up. No failures were observed among the 57 men who had not been sexually active. In addition, Katz *et al* observed that 15 (19%) of 79 chlamydia positive women who were treated with azithromycin were chlamydia positive 3 months after treatment.[3 5] Of these, 15 had not been sexually active and two (13.5%) were chlamydia positive. However, in the chlamydia screening studies, only one of 73 asymptomatic individuals, selected at random from the community, when treated with azithromycin 1 g immediately, were chlamydia positive on retesting 6 weeks after treatment.[7]

How can we explain these apparently contradictory observations and what implications do the studies by Golden *et al*[5] and Katz *et al*[6] have for the use of azithromycin or doxycycline in women? One possibility is that some of the women who denied sexual activity had indeed been sexually active and were thus re-infected. However, in order to account for a 8% failure rate in the 289 women being the result of re-infection, approximately 178 (62%) would have needed to have been sexually active given that the failure rate for women overall was 12.3% (164/1328). This seems unlikely; indeed behavioural variables such as number of sex partners, condom use, and failure to assure partner treatment were all associated with an elevated risk of re-infection in the study in question (Matthew Golden, personal communication), suggesting that the behavioural data participants

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Accepted for publication
24 February 2006

Abbreviations: BASHH, British Association of Sexual Health and HIV; MCC, minimal chlamydicidal activity; MIC, minimal inhibitory concentration; NAAT, nucleic acid amplification technique; NCSP, National Chlamydia Screening Programme; PCR, polymerase chain reaction

provided were substantially valid. The other, more likely, explanation is treatment failure.

With the majority of bacterial infections, if the micro-organism is not detectable following treatment, the individual is considered cured. It is assumed that an individual chlamydia negative 2–5 weeks after treatment is cured and that if *C trachomatis* is subsequently detected it represents a re-infection. However, there is considerable evidence from both in vitro and in vivo studies that *C trachomatis* can exist in a latent state, that is undetectable by culture, which can subsequently re-activate.[8] Thus, a duration of follow up longer than 2–5 weeks may be needed to establish that *C trachomatis* has been eradicated from the host.

In vitro it can be demonstrated that *C trachomatis* can enter a latent state under stressful conditions such as exposure to interferon γ (an important cytokine involved in cell mediated immunity), exposure to penicillins,[8] or amino acid starvation. This state allows *C trachomatis* to remain dormant but, on removing the stressful conditions, *C trachomatis* can be subsequently recovered from culture. How often this occurs in vivo is unknown but it may be an adaptive survival mechanism.[8] It is also not known how long this latent state can persist in vivo and whether removal of antimicrobial therapy can trigger reactivation.[9 10] There is also some evidence that latent infection may not be detectable, even using a NAAT, if only cells shed from the mucosal surface are sampled.[11 12]

Evaluation of the antimicrobial sensitivity of *C trachomatis* in vitro is problematic for technical reasons and there is no internationally agreed methodology.[9] The MIC (minimal inhibitory concentration) is lower than the MCC (minimal chlamydicidal activity) and it is unknown whether MIC is a biologically valid measurement. At high multiplicities of infection (load) in vitro persistence (latent infection) can often be demonstrated (heterotypic resistance). The subsequently recovered isolates do not possess antimicrobial resistance at low loads (homotypic resistance).[9]

Relatively few reports have described antimicrobial resistance in clinical isolates of *C trachomatis* associated with high level MICs irrespective of the innoculum size—that is, homotypic resistance.[9 10] One explanation for the apparent lack of antimicrobial resistance in *C trachomatis* may be the organism's unique developmental cycle. Since gene replication occurs isolated within an intracellular inclusion in an infected epithelial cell, acquisition of antibiotic resistance genes from other organisms would be difficult.[9] However, in vitro resistance has been demonstrated following the selective pressure from exposure to subinhibitory concentrations of antimicrobials—for example, fluoroquinolones and rifamycin.[13 14]

C trachomatis isolates from individuals with suspected treatment failure usually exhibit a heterotypic pattern of resistance, with only a small proportion of the population of organisms surviving.[9 10 15] This is observable when only large *C trachomatis* inocula were used and the surviving fraction does not show increased resistance. Although azithromycin has an excellent in vitro activity against *C trachomatis*, heterotypic resistance can be observed in vitro.[9 16] More recently, there is evidence to suggest that this pattern of heterotypic resistance may be a general phenomenon, observable in vitro with other antibiotics including doxycycline, when high infecting loads are present.[9] It is possible that this characteristic—that is, the survival of small numbers of chlamydial organisms in the presence of high levels of antimicrobials, has evolved because of selective pressure from frequent exposure to antimicrobials or this may be an innate characteristic of certain isolates, related to their ability to establish latent infection.[8–10]

There are only limited studies evaluating chlamydial load. Older studies, using culture, utilised the technique of inclusion colony forming units and these demonstrated that higher loads were detectable in women compared to men (although this may only reflect differences in the size of swabs used to sample the female cervix and male urethra), in those with inflammatory clinical disease compared to those without disease, in younger patients and in some serovars compared to others.[17 18] More recently, using real time polymerase chain reaction (PCR), a wide variation in chlamydial load has been observed with a mean of 1.3×10^5 plasmid copies per ml of vulvovaginal swab eluate (range 0–3.7×10^7), which was approximately 50 times higher than the mean of 2.8×10^3 plasmid copies/ml (range 0–8.7×10^5) for urine. Higher loads were also observed in those with inflammation.[19 20]

These observations suggest that the increased failure rate with longer follow up could be due not only to re-infection but also to re-activation of persistent latent infection as a result of heterotypic resistance associated with high chlamydial loads. While emergence of homotypic resistance is also a possibility, studies to date suggest that this would be unlikely. This phenomenon of heterotypic resistance could explain the apparently conflicting observations in treatment studies.[1 6 7] Failure would be expected to be more common in women than men, and in those with symptomatic disease as they probably have higher loads. This could also explain observations made during mass treatment with azithromycin of populations in trachoma endemic areas.[21 22] Treatment significantly decreased the proportion positive in the community and the load in the community. However, West *et al* observed that, whereas 91% of individuals with a low load at baseline, had no infection at 2 months, only 74% of participants with higher loads had no infection at 2 months after treatment ($p = 0.05$).[21] Finally, it is consistent with the observations of Hooton *et al* who observed that ciprofloxacin treatment was significantly more likely to fail to eradicate chlamydial infection in men with higher chlamydial loads compared to lower loads.[23]

While Katz *et al*[5] observed treatment failure with azithromycin and the majority of patients treated in the study by Golden *et al*[6] received azithromycin, it is probable that treatment failure also occurs with doxycycline 100 mg twice daily. Golden *et al* observed no difference in failure rates between these two treatment regimens and this is consistent with both short and longer term follow up studies comparing these regimens.[3 4 6] In the chlamydia re-infection study, there was no difference in the re-infection rates between women who received doxycycline ($n = 88$) and those who received azithromycin ($n = 325$) (HR 0.8, 95% CI: 0.4 to 1.4) (personal communication La Montagne).[4] Workowski *et al* followed 20 women for 5 months and observed that only one (2.5%) was chlamydia positive using an NAAT, and this was a re-infection.[24] Although 384 specimens were evaluated during the course of this study it is nevertheless a small study and it is likely that compliance with medication was high. However, there is evidence from in vitro work that doxycycline and azithromycin may have differential activity depending on whether *C trachomatis* is an acute infection or in a persistent (latent) state. Azithromycin is more efficacious in eradicating persistent infection than doxycline.[25] Although this study by Reveneau *et al*[25] suggests that doxycycline may be more efficacious in eradicating acute infection, in vivo studies suggest that this is not of clinical significance.[1] Resistance to tetracycline has been detected in vivo but appears to be rare.[26] Thus, given that heterotypic resistance is also demonstrable in vitro with doxycycline and that both long term and short term failure rates are similar to azithromycin 1 g, this phenomenon probably also occurs with doxycycline, particularly if compliance is poor.[9 27]

As there is evidence to show that the risk of developing pelvic inflammatory disease increases with each recurrence of *C trachomatis* infection, as does the risk of developing reproductive sequelae,[28 29] we should be concerned about the emerging evidence that, in some cases, the use of azithromycin 1 g immediately and probably also doxycycline may be suboptimal therapy in some women. The above observations suggest that recommended therapy[2] may fail in more than 5% of women, probably those with high loads.

Could a longer duration of treatment be more efficacious? Azithromycin is one of the most efficacious antimicrobials against *C trachomatis* in vitro and has a prolonged intracellular half life.[30] Dreses-Werringloer *et al* observed that after 8 days of exposure, viable *C trachomatis* was not recoverable in vitro although RNA could be detected up to 14 days.[16] Patton *et al* in the macaque PID model observed that an 8 day course of azithromycin resulted in complete eradication.[31] It therefore may be more appropriate to prescribe a longer course, although this may be associated with more side effects.[1] Such a regimen needs to be evaluated. The approach of giving two 1 g doses a given time apart should also be evaluated. The first dose would reduce the load and should make the second dose more efficacious. However, the time period between doses is unknown and from the evidence available may be longer than 1 week. Whether a longer course of doxycycline would be more efficacious is unknown. The study by Katz *et al*, who compared 7 days of tetracycline 500 mg four times daily with a 21 day regimen, suggests that a longer regimen may not be more efficacious.[32] However, this was a small study and it is not possible to exclude differences in re-infection between the groups as a potential source of bias.

To test the hypothesis that high chlamydia load predisposes to treatment failure with azithromycin 1 g and doxycline 100 mg twice daily for 7 days in women, would require a large study of approximately 600 women, with 300 in each treatment arm.[6] These women would need to remain sexually inactive for approximately 3 months following treatment.[5 6] Commercial assays are now available to measure chlamydial load using quantitative PCR. This technique has the advantage over culture, which requires considerable expertise, of being both accurate and reproducible and it can be undertaken by many laboratories, although it has the disadvantage in that it cannot distinguish live from dead organisms. This would need to be undertaken on all specimens pretreatment and on those chlamydia positive at follow up. These could then be typed molecularly in order to exclude re-infection from another source. In addition, those patients chlamydia positive at follow up should have isolates sent for culture, in order to obtain the MIC and MCC for the relevant antimicrobial. Such a study should also pay particular attention to collecting information on sexual activity following treatment in order to exclude re-infection as a possible cause of treatment failure. With the coming implementation of the National Chlamydia Screening Programme (NCSP) and the continuing recommendation in the forthcoming revised British Association of Sexual Health and HIV (BASHH) *Chlamydia trachomatis* clinical effectiveness guideline that no test of cure is required (P Horner and F Boag, personal communication),[2] such a study is urgently required and could be readily accommodated within the roll out of the screening programme.[33] If treatment failure rates of >5% are observed in some women then further studies using different treatment regimens would then be needed.[5 6] It may prove possible to identify those women at increased risk of treatment failure on clinical grounds, as symptoms are associated with higher loads (see before). Given the excellent in vitro efficacy of azithromycin,[9 25] its more favourable pharmacokinetic profile allowing less frequent dosing, and the findings from the animal study by Patton *et al*,[31] it may be more sensible to only use azithromycin for these studies.

Key messages

- The recent study by Golden *et al*[6] found an 8% (95% CI 5% to 11%) failure rate using recommended treatment regimens for uncomplicated genital *C trachomatis* infection in 289 women who had not subsequently been sexually active
- Conventional antimicrobial resistance has only rarely been observed with *C trachomatis* clinical isolates. However, at high multiplicities of infection (load) in vitro persistence can often be demonstrated to antimicrobials—heterotypic resistance. The subsequently recovered isolates do not possess antimicrobial resistance at low loads
- Chlamydia load varies considerably in vivo and there is evidence to support the hypothesis that heterotypic resistance could account for the findings of Golden *et al*
- Given the imminent roll out of the National Chlamydia Screening Programme and the consequences of persistent chlamydial infection in women this hypothesis urgently merits further investigation

In conclusion, although re-infection is probably the major cause of individuals retesting chlamydia positive following treatment there is emerging evidence that in some women this may also be the result of treatment failure with azithromycin or doxycycline, probably as a result of heterotypic resistance. Given the imminent roll out of the NCSP and the consequences of persistent chlamydial infection in women this hypothesis urgently merits further investigation. If this is indeed the case, new treatment regimens will need to be evaluated.

ACKNOWLEDGEMENTS

I would like to thank Jefferiss Trust for its support, and Dr Alan Herring and Professor Myra McClure for their helpful comments in preparing this manuscript.

Conflict of interest: none.

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