Effectiveness of single-dose azithromycin to treat latent yaws: a longitudinal comparative cohort study

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Summary

Background Treatment of latent yaws is a crucial component of the WHO yaws eradication strategy to prevent relapse and the resulting transmission to uninfected children. We assessed the effectiveness of single-dose azithromycin to treat patients with latent yaws.

Methods This population-based cohort study included children (age <20 years) living on Lihir Island, Papua New Guinea, with high-titre (rapid plasma reagin titre ≥1:8) latent or active yaws, between April, 2013, and May, 2015. Latent yaws was defined as lack of suspicious skin lesions or presence of ulcers negative for Treponema pallidum subsp pertenue on PCR, and active yaws was defined as ulcers positive for T pertenue on PCR. All children received one oral dose of 30 mg/kg azithromycin. The primary endpoint was serological cure, defined as a two-dilution decrease in rapid plasma reagin titre by 24 months after treatment. Treatment of latent yaws was taken to be non-inferior to that of active yaws if the lower limit of the two-sided 95% CI for the difference in rates was higher than or equal to −10%.

Findings Of 311 participants enrolled, 273 (88%; 165 with latent yaws and 108 with active yaws) completed follow-up. The primary endpoint was achieved in 151 (92%) participants with latent yaws and 101 (94%) with active yaws (risk difference −2.0%, 95% CI −8.3 to 4.3), meeting the prespecified criteria for non-inferiority.

Interpretation On the basis of decline in serological titre, oral single-dose azithromycin was effective in participants with latent yaws. This finding supports the WHO strategy for the eradication of yaws based on mass administration of the entire endemic community irrespective of clinical status.

Funding Newcrest Mining Limited and ISDIN laboratories.

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Introduction

As with venereal syphilis, patients with yaws can develop latent disease that is characterised by seroreactivity without other evidence of primary, secondary, or tertiary disease. Five to six people are estimated to have latent yaws for each case of active disease, and clinical relapse is possible for up to 5 years after infection. Clinical diagnosis of yaws has been challenged by the discovery that a substantial proportion of skin lesions contain no Treponema pallidum subsp pertenue on PCR, but contain DNA sequences specific to Haemophilus ducreyi or are caused by as-yet unknown pathogens. Some individuals with reactive yaws serology have skin lesions arising from another cause.

In 2012, a randomised controlled trial in Papua New Guinea showed that oral azithromycin was as effective as parenteral penicillin in the treatment of active yaws. On the basis of this finding, WHO adopted a revised strategy of treatment with single-dose azithromycin for all community members in yaws-endemic areas, irrespective of clinical status. Treatment of both active and latent yaws is crucial for the success of an eradication programme, and this strategy allows individuals with latent disease to be exposed to curative treatment. Despite implementation of pilot programmes in several equatorial rural provinces of Africa (Aziz A, personal communication) and the South Pacific, and plans to expand activities in those regions and worldwide, the efficacy of single-dose azithromycin to treat latent yaws remains to be established.

The objective of treating people with latent yaws is twofold: first, to prevent relapsing episodes and resulting transmission to uninfected children; and, second, to prevent progression of the infection to the destructive tertiary stage. Late latent syphilis is treated with benzathine benzylpenicillin for an extended period of time because this antibiotic is active only against dividing cells and organisms divide more slowly in the latent stage, although the validity of this rationale has not been assessed. Thus, single-dose azithromycin might be less effective in treating latent infection than active infection if the bacterium is less metabolically active.

In this study, we assessed the serological response of people with latent yaws to treatment with one dose of oral azithromycin. We compared the findings against those in people with active yaws.

Lancet Glob Health 2017; 5: e1268–74
Published Online October 26, 2017
http://dx.doi.org/10.1016/S2214-109X(17)30388-1
See Comment page e1172
Articles

Research in context

Evidence before this study
We searched PubMed, without language restrictions, for studies of the efficacy of single-dose oral azithromycin to treat latent treponemal disease published up to April 1, 2017, with the search terms “yaws”, “syphilis”, “latent”, and “azithromycin”. We identified four studies showing that azithromycin was efficacious in treating early latent syphilis in several regions (North America, Madagascar, Tanzania, and Taiwan). Additionally, studies in Congo, Ghana, Papua New Guinea, the Solomon Islands, and Vanuatu indicated that community-level prevalence of latent yaws fell substantially after mass treatment with azithromycin. In those studies, however, different samples of children were selected for each round of cross-sectional surveys, meaning that no study followed up a consistent group of participants. We did not identify any study that used serological monitoring to assess individual response to treatment of latent yaws with azithromycin.

Methods

Study design and participants
In the context of the yaws eradication programme of Lihir Island, Papua New Guinea, we did a prospective study that involved all children (age <20 years) with active or latent yaws who were residents of the island. Methods of the yaws eradication programme, including details of treatment and clinical assessment, have been previously described. Briefly, we administered mass azithromycin treatment in April, 2013, and repeatedly assessed outcomes in about 16000 people every 6 months for about 3-5 years.

Participants attended clinical surveys, during which those with ulcers suspected of being due to active yaws were assessed for inclusion in the study. People with a reactive T pallidum haemagglutination assay (TPHA) and rapid plasma reagin (RPR) titre of 1:8 or greater at the time of screening were eligible for the study. We excluded people with RPR titres of 1:2 or 1:4 because many children in high endemic areas have a persistently positive low-titre RPR results after successful treatment (ie, serofast status) that cannot be distinguished from true treponemal infection. The criterion for the diagnosis of active yaws was ulcers positive for T pertenue on PCR, and for the diagnosis of latent yaws was ulcers negative for T pertenue. Asymptomatic individuals (no suspicious skin lesions) from randomly selected villages who were assessed and enrolled during baseline serosurveys of the eradication programme were also included.

We classified participants with dual positivity on TPHA and RPR into three groups, on the basis of PCR testing: group 1 included asymptomatic people with no ulcers and who were not tested with PCR; group 2 included people with ulcers negative for T pertenue on PCR; and group 3 included people with an ulcer and PCR evidence of active yaws. Groups 1 and 2 constituted the latent yaws study group. The Medical Research Advisory Committee of Papua New Guinea approved the study protocol. The Medical Research Advisory Committee of Papua New Guinea approved the study protocol (number 12.36). All eligible people were informed of the study’s aims, procedures, and associated risks and provided informed consent (children <15 years gave verbal consent and their parents or guardians provided written consent).

Procedures

All participants with skin ulcers underwent clinical, serological, and PCR assessments, were treated with one directly observed oral dose of 30 mg/kg azithromycin, and were followed up in village community outreach clinics for repeated RPR testing at 6, 12, and 24 months. Follow-up was discontinued when results showed serological cure. Asymptomatic participants also received one oral dose of 30 mg/kg azithromycin and were followed up at 12 and 24 months.

The serological reactivity was determined at the Lihir Medical Centre laboratory by RPR and TPHA testing (both Human Diagnostics Worldwide, Wiesbaden, Germany). To calculate RPR titre, a blood serum sample was diluted in serial ratios until there was no longer antibody reactivity. Real-time (rt)PCR specific for T pertenue and H ducreyi was done on all lesional swab samples at the University of Washington laboratory, Seattle, WA, USA. Three primer sets for three independent T pallidum gene targets—tp0548, tpN47 (also known as
tp0574), and a pertenue-specific region of trpL (also known as tp1031)—were PCR amplified to detect the presence of T pallidum DNA and to confirm the subspecies, as has been described previously. We used Taqman rtPCR targeting the 16S rRNA gene to identify H ducreyi.

Definitions
The primary endpoint of the study was serological cure at 24 months, defined as a decrease in the RPR titre by at least two dilutions (fourfold) at any time during the study (eg, change from 1:16 at baseline to 1:4). Participants not cured at 24 months were classified either as having treatment failure, defined as at least two dilutions (fourfold) increase in RPR titre (eg, change from 1:16 at baseline to 1:64), or having non-significant change, defined as either no change in titre or a decrease or increase of one dilution (twofold) from baseline (eg, change from 1:16 to either 1:8 or 1:32), based on the 24-month RPR titre. The outcome categories were mutually exclusive. The secondary endpoint was the proportion of participants with seroreversion to non-reactive at 24 months (ie, no antibodies detectable by RPR testing).

Statistical analyses
We calculated that a sample size of 165 participants in the latent yaws group and 108 in the active yaws comparator group (sampling ratio 1:5) would give a power of 98% to test the hypothesis of non-inferiority. This sample size accounted for an expected efficacy of 91.5% in the latent yaws group and of 93.5% in the active yaws group, a non-inferiority margin of 10%, and a one-sided type I error rate of 0.05.

We included in the primary analysis participants who were treated and completed the study procedures for outcome assessment at 24 months. Data on participants who were lost to follow-up before serological cure were censored at the time of the last follow-up visit and were not included in the primary analysis.

To analyse the primary endpoint, we estimated the two-sided 95% CIs for the difference in proportions of participants with serological cure by subtracting the active yaws group values from the latent yaws group values. Treatment of latent yaws was deemed to be non-inferior to that of active yaws if the lower bound of the CI was higher than or equal to −10%. We used the same method to analyse the secondary endpoint. We did subgroup analyses to investigate the association between serological cure and time of retesting by use of differences in proportions or mean baseline RPR titre, assessed with the Student’s t test of geometric means with serum RPR titre as a continuous variable. We did all statistical analyses with Stata version 14.1. This

Figure 1: Study profile
TP = Treponema pallidum subsp pertenue. TPHA = T pallidum haemagglutination assay. RPR = rapid plasma reagin.
study is registered with ClinicalTrials.gov, number NCT01955252.

**Role of the funding source**

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

**Results**

Of 1155 individuals assessed for eligibility, 484 were asymptomatic (group 1), 483 had skin ulcers negative for *T pertenue* on rtPCR (group 2), and 188 had skin ulcers positive for *T pertenue* on rtPCR (group 3). Among these, 311 children had a reactive TPHA and an RPR titre of at least 1:2 or 1:4 in groups 2 (n=54) and 3 (n=21), who were excluded from the main analysis. In group 2, 28 (52%) participants achieved a two-dilution decrease in RPR titre or seroreversion at 24 months, compared with 15 (71%) in group 3 (risk difference –19·6%, 95% CI –43·0 to 3·9). Some individuals with baseline RPR titres of 1:2 or 1:4 who had persistently unchanged RPR titres at 24 months were probably actually serofast, which supports the appropriateness of excluding this group of participants from the analyses of serological cure at 24 months. 252 patients who were classified as having serological cure and 21 as having treatment failure or non-significant change outcomes at 24 months.

The risk difference for serological cure between the latent versus the active yaws groups met the prespecified criteria for non-inferiority (table 2). In the sensitivity analysis of participants who were tested three times (ie, excluding group 1), non-inferiority was maintained (83 [93%] vs 101 [94%], risk difference –0·3%, 95% CI –7·2 to 6·7). Additionally, the proportions of participants with serological cure at 24 months were similar in groups 1 and 2 (68 [90%] vs 83 [93%], p=0·383), which supports the adequacy of combining asymptomatic and PCR-negative participants in the latent group. 14 participants with latent yaws did not achieve serological cure (table 3). Three or four dilution decreases in RPR titre were seen frequently in both the latent yaws and active yaws groups (table 3). The proportion of participants with seroreversion was significantly lower in the latent yaws group than in the active yaws group (table 2). We did a post-hoc analysis of individuals with baseline RPR titres of 1:2 or 1:4 in groups 2 (n=54) and 3 (n=21), who were excluded from the main analysis. In group 2, 28 (52%) participants achieved a two-dilution decrease in RPR titre or seroreversion at 24 months, compared with 15 (71%) in group 3 (risk difference –19·6%, 95% CI –43·0 to 3·9). Some individuals with baseline RPR titres of 1:2 or 1:4 who had persistently unchanged RPR titres at 24 months were probably actually serofast, which supports the appropriateness of excluding this group of patients from our analysis.

At retesting visits, the latent yaws group included only group 2 participants at 6 months, but group 1 and 2 participants at 12 months. The proportions with serological cure did not differ significantly between the latent and active yaws groups in the subgroup analysis of time of retesting, either at 6 months (58 [83%] vs 59 [88%], risk difference –5·2%, 95% CI –17·0 to 6·6) or at 12 months (125 [86%] vs 74 [90%], –4·0%, –12·6 to 4·5; figure 2A). The proportion of participants with seroreversion was lower in the latent yaws group than in the active yaws group at 6 months (12 [17%] vs 30 [45%]; risk difference –27·6%, 95% CI –43·0 to –12·8) and 12 months (47 [32%] vs 44 [54%], –21·2%, –34·5 to –8·0; figure 2C).

Mean baseline RPR titre was 40·1 (SD 43·3) in participants with serological cure, compared with 34·9 (24·0) in participants who did not achieve serological cure (p=0·721). We saw no difference in serological response at 24 months between participants with latent or active yaws by baseline RPR titre (figure 2B). The mean baseline RPR titre was 33·3 (SD 35·4) in participants...
with seroreversion compared with 43.8% (SD 43.8%) in participants without seroreversion (p=0.034). The proportion of participants with seroreversion at 24 months was lower among those with latent yaws than among those with active yaws by RPR titre at baseline (figure 2D).

Discussion
The primary endpoint of serological cure at 24 months was seen in 92% of patients with latent yaws after treatment with single-dose azithromycin, which was similar to that among participants with rtPCR-confirmed active yaws. These results expand on the data showing efficacy with 30 mg/kg single-dose azithromycin for treatment of early syphilis, including in the early latent stage. Before this study, mass treatment with azithromycin had been shown to reduce the prevalence of latent yaws significantly at the community level. People with latent yaws, however, had not been consistently and prospectively followed up over a long period of time to monitor serological cure at the individual level.

The RPR titre data indicate that successful treatment for active and latent yaws often results in three or four dilution decreases, but seroreversion was seen with this non-treponemal test in less than 50% of participants with latent yaws. As reported for syphilis, participants with high baseline titres in this study had greater declines than did those with lower titres at baseline, but were less likely to have seroreversion. We stress that non-treponemal tests can show reactivity at low titres in a high percentage of people, even a long time after treatment, and, therefore, do not necessarily indicate untreated yaws. Similar persistent reactivity at low titres is seen after treatment of syphilis, and is referred to as serofast status. This evidence supports the inclusion of serosurveys in yaws eradication programmes to assess interruption of yaws transmission at least 24 months after mass treatment, alone or with the use of an RPR titre cutoff below which further testing is needed before serology is classified as positive.

Our study has several limitations. First, the RPR test is read by naked eye and there is a degree of inter-observer variability in interpretation of test results that can lead to variation in RPR titre of plus or minus one dilution. We attempted to minimise variation by having all RPR testing done by one technician at a central laboratory attempting to minimise variation by having all RPR testing done by one technician at a central laboratory. Second, because of the observational nature of our study design, there was no untreated control group. As the duration of untreated syphilis increases, titres might decline by two or more dilutions. A similar effect could have contributed to the results for yaws in our study. Additionally, there was no control group of participants treated with benzathine benzylpenicillin. We did, however, include a comparator group of children with active yaws infection, and a study of single-dose azithromycin compared with benzathine benzylpenicillin for active yaws showed non-inferiority. Third, by excluding people with baseline RPR titres of 1:2 or 1:4, the latent sample might be biased in favour of early latent infection or people between the primary and secondary stages. Serological cure at 24 months was seen in only 52% of participants with low-titre RPR latent yaws, which might reflect stable non-treponemal antibody titres in the serofast state. However, we cannot rule out that the lower serological cure exhibited was related to infections later in the natural history. This pattern, though, would not affect the WHO yaws eradication programmes because late latent yaws, in which lesions are not present, is not an important contributor to incident infections, but it would have implications for individuals’ health and clinical management. Finally, group 1 had a different follow-up schedule from groups 2 and 3 (two vs three occasions for testing) and, therefore, serological cure might have been underestimated in group 1. Although this difference could bias the non-inferior result towards inferiority for treatment of latent yaws, we were still able to show non-inferiority. Furthermore, non-inferiority was maintained in a sensitivity analysis of only groups 2 and 3. Of note, in people with latent yaws (or syphilis) it can take 12–24 months for the full amplitude of RPR titre fall to be expressed after successful
treatment and, therefore, serology at 12 and 24 months is more reliable than assessment at 6 months, which group I missed.

In common with other yaws-endemic regions studied, we found that a substantial proportion of skin lesions clinically attributed to yaws contained *H. ducreyi* DNA.1 Isolates of *H. ducreyi* obtained from skin lesions of children in Vanuatu and Samoa were sensitive to azithromycin in vitro,23 and the antibiotic is recommended for the treatment of sexually transmitted *H. ducreyi* infections.24 On Lihir Island, 2 weeks after treatment with azithromycin, around 90% of all ulcers containing *H. ducreyi* were totally re-epithelialised or greatly improved.25 Hence, azithromycin has the double effect of treating *H. ducreyi* ulcers and latent yaws.

Failure of targeted treatment of people with active yaws and their contacts with penicillin by WHO and UNICEF in the 1940s and 1950s was thought to be due to continued untreated asymptomatic infection because of the tendency for untreated latent yaws to relapse early.26,27 Treatment of latent disease is, therefore, a crucial part of persistent transmission of the disease. More latent yaws support relapse from untreated latent yaws, and their contacts with penicillin by WHO and UNICEF, continued cooperation. We thank Sergi Gavilán, Barcelona Institute for Global Health, for knowledge management, liaison between institutions, and acquisition of funding. This study was funded by Newcrest Mining and ISDIN laboratories. The laboratory work was supported by the US Public Health Service National Institutes of Health (grant R01AI42143 to SAL), and WHO provided generic azithromycin.

References


