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Optimisation of treatments for oral *Neisseria gonorrhoeae* infection: Pharmacokinetics Study (STI-PK project) – study protocol for non-randomised clinical trial

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ABSTRACT

Introduction *Neisseria gonorrhoeae* infections are common and incidence increasing. Oropharyngeal infections are associated with greater treatment failure compared with other sites and drive transmission to anogenital sites through saliva. Gonococcal resistance is increasing and new treatments are scarce, therefore, clinicians must optimise currently available and emerging treatments in order to have efficacious therapeutic options. This requires pharmacokinetic data from the oral cavity/oropharynx, however, availability of such information is currently limited.

Methods and analysis Healthy male volunteers (participants) recruited into the study will receive single doses of either ceftriaxone 1 g, cefixime 400 mg or ceftriaxone 500 mg plus 2 g azithromycin. Participants will provide samples at 6-8 time points (treatment regimen dependent) from four oral sites, two oral fluids, one anorectal swab and blood. Participants will complete online questionnaires about their medical history, sexual practices and any side effects experienced up to days 5–7. Saliva/oral mucosal pH and oral microbiome analysis will be undertaken. Bioanalysis will be conducted by liquid chromatography-mass spectrometry. Drug concentrations over time will be used to develop mathematical models for optimisation of drug dosing regimens and to estimate pharmacodynamic targets of efficacy.

Ethics and dissemination This study was approved by Royal Melbourne Hospital Human Research Ethics Committee (60370/MH-2021). The study results will be submitted for publication in peer-reviewed journals and reported at conferences. Summary results will be sent to participants requesting them. All data relevant to the study will be included in the article or uploaded as supplementary information.

Trial registration number ACTRN12621000339853.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This is the first comprehensive study to collect pharmacokinetic (PK) data of drugs used to treat gonorrhoea in the oral space from four oral sites, two oral fluids and blood. The data are complemented by data at the anorectal site for comparison.
⇒ This data will inform optimisation of drugs to treat oropharyngeal gonorrhoea and develop methods to apply to drugs in phase 2 or 3 randomised controlled clinical trials.
⇒ While we did not obtain true tissue samples (eg, via biopsies) but rather swabs of surface mucosa, this will still allow examination of drug distribution by oral cell type, for an infection that is primarily at the epithelial surface.
⇒ The study does not include women or those with oropharyngeal gonorrhoea infections.
⇒ As we only include healthy volunteers, there are no data on bacterial minimum inhibitory concentrations (MICs) to assess antimicrobial resistance and unable to generate real-world pharmacodynamic (PD) data, but we will estimate PK/PD target achievement based on the PK data and models using various *Neisseria gonorrhoeae* MICs.

INTRODUCTION

Neisseria gonorrhoeae (NG) is the second most common bacterial sexually-transmitted infection (STI) globally.[1] Over the last 10 years, NG infections have increased markedly—by 370% in Australia,[2] 75% in the USA[3] and 250% in the UK.[4] Oropharyngeal NG is common with a prevalence of approximately 2%[5] and 5%[5] among heterosexuals and men who have sex with men attending clinical services, respectively. Oropharyngeal infections are important because (1) cure rates

at the oral site are up to 20% lower than at the genital site[6]; (2) play a major role in transmission in the population through oral sex and use of saliva[7] and (3) they are more likely to facilitate the development of antimicrobial resistance (AMR).[8] NG has now developed resistance to all classes of antibiotics recommended for gonorrhoea treatment[9] and in 2017, the WHO declared AMR NG as an urgent global threat.[10] Therefore, ensuring continued access to effective treatments remains a global challenge.

There is a scarcity of pharmacokinetic (PK) data for antibiotics in the oral cavity or oropharynx, and it remains unclear if lower oropharyngeal NG cure rates are due to inadequate tissue concentrations of antibiotics at the oral sites where NG grows. PK data for NG treatments in the oropharynx are currently only available for the tonsils.[11] However, it is not well understood where NG infects the oropharynx or oral cavity. Further, there are no PK data available for the mouth for emerging NG treatments currently in phase 2–3 randomised controlled trials (RCTs). It is unlikely that any new STI drugs will reach the market in the near future[12] as the few drugs in current phase 2–3 trials are either producing estimates below the CDC efficacy criteria of 95%[13] for treating oral NG or have not been appropriately evaluated for oral infection. This does not provide much optimism unless drug therapy can be optimised by changing the dosing regimen. However, optimisation needs PK data at the site of infection, that is, oral tissue.

We are conducting a non-randomised trial to generate comprehensive human PK data for oral NG treatments. These data can then be used to optimise available treatments and improve their efficacy to break the ongoing transmission and development of AMR. This paper describes the study methodologies for collecting PK data on currently recommended antimicrobial treatments for oropharyngeal NG (ceftriaxone 1 g, cefixime 400 mg and ceftriaxone 500 mg plus 2 g azithromycin) from human blood, four oral sites and two oral fluids. Given the scarcity of PK data for the anorectum, we will also take the opportunity to measure antibiotic concentrations in the anorectum, although cure rates for anorectal NG are much higher compared with oral NG.

Research aim and hypothesis

The primary aim of this study is to determine the PK properties of antibiotics to treat NG in the oral cavity (tongue, gingival crevicular fluid (GCF), saliva) and oropharynx (cheeks, tonsils, posterior pharyngeal wall)—collectively referred to as 'oral' in this protocol. Our secondary aims are to (1) determine pharmacodynamic (PD) targets at the oral site; (2) measure pH in the oral site; (3) assess the impact of the treatments on the oral microbiome and (4) measure antibiotic concentrations in anorectal mucosal tissue. This study will specifically explore the PK of recommended oral NG treatments at the time of the study design, namely single doses of ceftriaxone 1 g,[14] ceftriaxone 500 mg plus 2 g azithromycin[15] and cefixime 400 mg.[16 17] These drugs have been selected for evaluation because they represent the main antibiotics likely to be used prospectively and amenable to optimisation.

Our hypothesis is the PK properties of drugs vary by the site of infection resulting in differences in treatment efficacy, especially at non-urogenital sites such as at the oral and anorectal site. Therefore, different treatment regimens are needed for the optimal treatment of non-urogenital NG infections.

OUTCOMES

Primary outcome

Our primary outcome is to estimate PK data for each antibiotic, including: drug concentrations (total and protein unbound in blood and saliva) (C), peak concentrations (Cmax), time to reach Cmax (Tmax), area under the concentration-time curve (AUC - first 24 hours: $AUC_{0\text{-}24}$; total: $AUC_{0\text{-}\infty}$), absorption rate constant (Ka), clearance (CL), volume of distribution (Vd) and half-life ($T_{1/2}$). These data will be estimated in blood (venous or peripheral blood), tissue/mucosa (oral and anorectal), saliva and GCF.

SECONDARY OUTCOMES

The magnitude of the PK/PD targets will be estimated by calculating (1) the percentage of time during which the protein unbound drug concentration exceeds the minimum inhibitory concentration (MIC) (%*f*T>MIC) for cephalosporins (2) the ratio of the AUC unbound drug concentration-time curve to the MIC (*f*AUC/MIC) for azithromycin and (3) the ratio of the maximum unbound drug concentration to the MIC (*f*Cmax/MIC) for azithromycin.

We will also measure the pH of the oral mucosa and saliva, saliva flow rate and oral microbiome changes. We will obtain PK data for each antibiotic in anorectal mucosa to compare to those at the oral sites.

METHODS AND ANALYSIS

Study design and setting

This is a non-randomised, open label antibiotic trial among healthy volunteers. The trial will be conducted in an urban general practice in Victoria, Australia.

Duration of study

For those receiving monotherapy with ceftriaxone 1 g or cefixime 400 mg, the study requires three in-person visits (over 3 days) and for those receiving dual therapy with ceftriaxone 500 mg plus 2 g azithromycin, five in-person visits (over 14 days) are required. Online self-administered questionnaires are completed during and after these visits. Recruitment commenced in April 2022 with anticipated completion by June 2023.

PARTICIPANTS

Recruitment

Healthy men who self-report they are free of STIs will be recruited through advertising on social media (including

Twitter and Facebook), University of Melbourne news emails and word of mouth. Interested participants will be contacted by a member of the research team to discuss the study by telephone. Those eligible will be scheduled to attend the general practice in person where written informed consent is obtained. Women will be excluded from the initial recruitment until after the preliminary results are obtained from men to permit refinement of sampling methods.

Inclusion and exclusion criteria

Men aged 18 years or older will be eligible if they have adequate comprehension to give informed consent, are able to attend all follow-up visits, have an Australian Medicare card (Australia's national insurance scheme for healthcare) and have received at least three doses of COVID-19 vaccination. Those who have used antibiotics in the 4 weeks prior to the baseline visit, have widespread mucosal ulcerations by clinical examination, transgender people and people living with HIV with CD4 counts <250 cells/mm^3 will be excluded.

Treatment and allocation

Three antibiotic regimens are being evaluated and include those recommended for treating oropharyngeal NG at the time of the study in Australia or internationally, that is, (1) ceftriaxone 1 g (Ceftriaxone-AFT, China) reconstituted in 3.5mL 1% lignocaine (Pfizer, Australia) as a single dose by intramuscular injection; (2) ceftriaxone 500 mg reconstituted in 2mL 1% lignocaine as a single dose by intramuscular injection plus 2 g oral azithromycin tablet (1 g followed by 1 g 6–12 hours later, taken with food)[18] (Sandoz, Australia) or (3) oral cefixime 400 mg capsule as single dose, taken on an empty stomach (Denvar, Spain).

The second 1 g azithromycin dose will be administered after the 6-hour sample has been taken (during the first visit) if the participant is not experiencing significant adverse events. If they are, they will be asked to take the second dose before they go to sleep (approximately 9pm or 12 hours after the dose).

Treatments will not be randomly allocated, rather they will be allocated in batches until the required sample size is obtained for each regimen, with the first treatment investigated being ceftriaxone 1 g.

Reimbursement

Each participant will be reimbursed a maximum of $A1000 for reasonable time and expenses (food and transport)—$A500 at the conclusion of the baseline visit and a further $A500 at the conclusion of the final in-person visit.

Specimen collection and measurements

For each participant, antibiotic concentrations will be measured from four oral sites, two oral fluids and blood. An anorectal swab will also be collected.

Specimen collection from participants is summarised below and in table 1.

Oral swabs/curettes specimen collection for PK and PD analysis

(A) tonsils (tonsil and posterior tonsillar pillar) by swiping both areas three times with a FloqSwab (552 c; Copan, France), (B) from the posterior pharyngeal wall by swiping the site six times with FloqSwab and (C) 15 swipes of (1) the buccal mucosa of each cheek and (2) lateral sides of tongue using a dermal curette (4 mm; Kai Medical, Japan).

To minimise the gag reflex, participants are asked to open their mouth wide, inhale and then gently hold their breath before sampling.

Oral fluids specimen collection for PK and PD analysis

All participants are asked to rest their mouth (no eating, drinking, chewing, smoking, etc) for a minimum of 30 min prior to the collection of saliva and GCF. A 1 mL of saliva will be collected by dribbling into a cup. GCF will be collected by placing two PerioCol strips (Oraflow, USA) at the central or lateral incisors and leaving in place for 1 min.

Blood collection for PK and PD analysis

(A) 5 mL of blood will be collected via venepuncture and plasma obtained by centrifugation at 3500 rpm (2500× g) for 15 min (BD Vacutainer 102 IU lithium heparin, ref. 367885), (B) 10 µL of finger prick blood will be collected using volumetric absorptive microsampling (VAMS; Neoteryx Mitra) in duplicate and (C) 10 mL of whole blood to measure baseline blood biochemistry for analysis of renal and liver function (BD Vacutainer 171 IU lithium heparin, ref. 367375) and haematocrit (BD Vacutainer 5.4 mg EDTA, ref. 367838) to be used in PK optimisation estimations.

Specimen collection to evaluate oral microbiome

Sample will be collected by swabbing the posterior oropharynx, its side walls and tonsillar crypts with a total of six swipes using an Eswab (Copan, France).

Anorectal swab

Anorectal swab will be self-collected by inserting a FloqSwab 5 cm into anorectum and rotating gently for 5 s.

Sampling and data collection times

Collected samples and pH measurements will be taken before (baseline), 1-2, 4, 6, 24 and 48 hours after the antibiotic dose. Samples taken at baseline to the 6-hour time point will be taken during the same visit. For the ceftriaxone 500 mg plus 2 g azithromycin arm, the first postdose sample will be taken after the ceftriaxone and first 1 g dose of azithromycin. For ceftriaxone and azithromycin dual therapy, additional samples will be taken at day 7 and 14 days postdose due to the long half-life of azithromycin (table 1).

Patient and public involvement

No patient involved. Summary results will be sent to participants who consent to receiving them.

Table 1 Summary of sampling frame

			Sampling times (post dose)								
Site	Sample type (in order of sample collection)	Screening for eligibility	0 hour* (Baseline, before dose)	1–2 hours*	4 hours*	6 hours*	day 1	day 2	days 3–5	day 7†	day 14†
	Informed consent	X									
	Baseline survey			X							
	Follow-up surveys					X	X	X	X	X	X
Oral	Saliva flow rate		X								
	Saliva—pH		X	X	X	X	X	X		X	X
	pH of buccal mucosa and tongue		X	X	X	X	X	X		X	X
	Saliva—drug		X	X	X	X	X	X		X	X
	GCF		X	X	X	X	X	X		X	X
	Oral swabs (four sites)		X	X	X	X	X	X		X	X
	Microbiome		X	X	X	X	X	X		X	X
Bloods	VAMS		X	X	X	X	X	X		X	X
	Blood —full blood count and biochemistry, LFT		X								
	Blood for plasma and whole blood for VAMS		X	X	X	X	X	X		X	X
Anorectum	Swab for drug level		X	X	X	X	X	X		X	X

*Samples taken at times 0–6 hours are all taken during the same visit that is, during the 'day stay'.
†Days 7 and 14 for ceftriaxone 500 mg plus 2 g azithromycin arm only.
GCF, gingival crevicular fluid; VAMS, volumetric absorptive microsampling; LFT, liver function test.

Participant data

Men's demographics, weight, medical history (smoking status, malabsorption conditions, concurrent medications, STIs and meningococcal vaccination status in the past year), sexual practices, recreational drug use and oral health will be recorded at recruitment. During the follow-up period, men will be asked if they had oral or anal sex prior to each in-person visit and any antibiotic side effects (nausea, vomiting or diarrhoea).

Adverse events reporting

We do not expect any severe adverse events, as these drugs have been widely used for decades and their side effect profiles are well-established. Daily mobile SMS will be sent to each participant to collect any nausea, vomiting or diarrhoea for 5 days postdose for all antibiotics, except for participants on ceftriaxone with azithromycin who will receive SMS for 7 days due to the longer half-life of azithromycin.

Study survey data will be collected and managed by using REDCap electronic data capture tools hosted at The University of Melbourne.

ANALYSIS

Laboratory analysis

Specimen analysis

All oral swabs/curettes and PerioCol strips will be placed in 2 mL tubes containing 0.5–1 mL 100% methanol and stored immediately at −20°C until delivery to the laboratory where they will be stored at −80°C until analysis. Saliva and VAMS will be stored neat in 2 mL tubes. Drug concentrations will be estimated using liquid chromatography-mass spectrometry performed to industry standard with pre-established batch acceptance criteria applied to ensure the reliability of the resulting data.[19] Protein unbound ('free') drug will be measured in plasma and assumed from saliva as only free drug distributes into saliva.

pH measurements

The pH of saliva and oral mucosa will be measured as studies have reported increases in some antimicrobial MICs with lowering pH and pH affects the degree of drug ionisation and penetration into cells.[11] All participants will be asked to rest their mouth for at least 30 min prior to saliva and oral mucosal pH measurements.

Saliva pH will be measured by a drop of saliva into the Lacquatwin pH meter (pH22, Horiba, USA). The surface pH of the side of the tongue and buccal mucosa will be measured by placing a flat head pH meter (Hanna, HI99171; USA) against the oral mucosal surface as per previous methods.[20]

Specimens collected for saliva flow rate: At baseline, after resting the mouth for at least 30 min, saliva will be collected into a cup over 1 min and then the volume collected measured (mL/min).

Sample size estimation

We have used optimal sampling design (OSD) methodologies using published PK data to determine the number of subjects and the number and timing of samples needed for each drug to provide sufficiently precise estimates of the PK model parameters. Our calculations were based on the number needed for measuring PK in blood samples because there are no published data available for tissue samples at our infection sites. Using OSD methods and taking into consideration recruitment challenges due to the requirement for intensive sampling among healthy volunteers and COVID-19 restrictions, up to 20 people per drug is considered sufficient and in line with previous PK studies in the mouth.[21]

PK analysis

Non-linear mixed-effects modelling will be performed using the FOCE+I algorithm in the NONMEM software. For each drug, the plasma concentration-time profiles will be modelled first. One-compartment, two-compartment and three-compartment models will be evaluated, with linear, saturable or mixed-order elimination. To describe absorption, first-order and zero-order, simultaneous or sequential first-order and zero-order processes will be tested. Profiles in saliva and oral swabs/curettes will be subsequently included. An MC-PEM algorithm, minimal physiologically-based PK modelling approach[22] and/or three-stage hierarchical Bayesian method may be considered as needed.[23] Interindividual variability for the population PK parameters will be estimated where possible. Individual (post hoc) PK parameter estimates will be graphed against biological subject characteristics (eg, weight, creatinine clearance) for initial exploration of potential covariate relationships. Covariates will be formally evaluated by forward inclusion followed by backwards elimination. Model selection will be based on goodness-of-fit plots, visual predictive checks, the normalised prediction distribution error, the log-likelihood ratio test (for nested models; Akaike information criterion for non-nested models) and biological plausibility. For each drug, the Cmax, Tmax, elimination half-lives and AUC ($AUC_{0\text{-}24}$, $AUC_{0\text{-}\infty}$) will be calculated from the individual estimated PK parameters or read from the individual fitted PK profiles. For the PK/PD indices, the magnitude of %*f*T >MIC will be estimated for ceftriaxone and cefixime. The magnitudes of *f*AUC/MIC and *f*Cmax/MIC will be estimated for azithromycin. The NG MICs used for PK/PD target attainment of ceftriaxone and cefixime will be 0.002, 0.004, 0.008, 0.015, 0.03, 0.06, 0.125, 0.25, 0.5, 1.0 and 2.0 mg/L. NG MICs used for azithromycin will be 0.125, 0.25, 0.5, 1.0, 2.0, 4.0 and 8.0 mg/L.

Microbiome analysis

Microbiome analysis will be used to understand the impact of antibiotics on oral microbiota and to examine any associations with drug concentrations, since human gut biota has been shown to modulate the efficacy of drugs.[24]

Microbiome analysis will be undertaken as previously described.[25] DNA will be extracted from tonsillar samples using the QIASymphony PowerFecal Pro kit (Qiagen). Extracted DNA will be used to generate an amplicon-based library using primers that amplify the V4 region of the 16S rRNA gene: 515F (59-GTGYCAGCMGCCGCGGTAA-39) and 806R (59-GGACTACNVGGGTWTCTAAT-39). Libraries (biological samples, as well as positive and negative controls) will be sequenced on an Illumina MiSeq instrument (Illumina, San Diego, California, USA) with a 2 by 150 bp run through Doherty Applied Microbial Genomics at The Peter Doherty Institute for Infection and Immunity, University of Melbourne.

Demultiplexing and trimming of sequencing reads will be conducted using the online tool Qiita (https://qiita.ucsd.edu). Reads will be demultiplexed using split libraries FASTQ and trimmed to 150 bp (V.QIIMEq2 1.9.1). DADA2 V.1.16.0 will be used to quality-filter the sequence data, infer amplicon sequence variants (ASVs) and remove chimaeras. DADA2 and a DADA2 formatted version of the Silva reference database (v138) will be used to assign taxonomy down to the genus level. We will visually compare the oropharyngeal microbiota composition as per schedule in Table 1 by principal component analysis of centre-log ratio-transformed ASV level sequence data, using mixOmics (V.6.12.1). PERMANOVA based on the Bray-Curtis distance will be used to test for differences in the overall structure of the oropharyngeal microbiota. Bacterial diversity will be calculated on ASV data using the Shannon diversity index using vegan V.2.5–7. Changes in bacterial diversity following treatment will be used to assess using the Wilcoxon signed-rank test. We will investigate differences in the baseline oropharyngeal microbiota composition between individuals with and without specific characteristics/factors collected in the baseline survey.

ETHICS AND DISSEMINATION

Ethics approval

This study was approved by Royal Melbourne Hospital Human Research Ethics Committee (60370/MH-2021). The study is based on voluntary participation and a written informed consent process.

Clinical trial registration

The study is registered with the Australian drug regulator, The Therapeutic Goods Administration (Clinical Trial Notification CT20006 CT-2021-CTN-00571–1V2) and with the Australian New Zealand Clinical Trials Registry (Trial ID ACTRN12621000339853)

Dissemination plans

The study results will be submitted for publication in peer-reviewed journals and reported at national and international conferences. These data will be used to inform other drug optimisation studies or modelling to prevent NG AMR. Summary results will be sent to participants who consent to receiving them. All data relevant to the study will be included in the article or uploaded as supplementary information in future papers).

DISCUSSION

Treatment options for gonorrhoea are diminishing as NG becomes increasingly resistant—particularly at the oropharyngeal site. The primary objectives of STI treatment are to maximise cure, minimise drug toxicity and avoid induction or selection of AMR. Knowledge of the PK characteristics of drugs can guide development of treatment regimens. Simply measuring the concentrations in tissue and blood as is done in most trials of new NG treatment, is not enough. This trial will generate the most comprehensive PK data available today from four oral sites, two oral fluids and blood. It will also estimate PK/PD target achievements based on the PK data and model. It will do this by using new and validated methods including the use of blood VAMS, which will allow bloods to be taken in the home setting. The data and methods will inform optimisation of drugs in phase 2 or 3 RCTs and Hollow Fibre Infection Models.[26 27]

Oropharyngeal NG is a major driver of ongoing transmission, contributing to 50% of new NG infections in the anorectum through saliva in some settings,[28] and it can cause serious reproductive sequelae (eg, pelvic inflammatory disease) by being passed to female genitalia via oral sex.[29] As concerns for global AMR increases with few antimicrobials for STIs in development,[30] clinicians have little choice but to maximise the use of currently available treatments. One approach is to optimise currently available antibiotics, but this requires an understanding of the PK of these drugs in the target population, including their distribution to the site of infection. Only drug that is unbound to protein ('free' drug) is pharmacologically active, so measuring this is critical.

Even though a drug reaches adequate concentrations in tissue, this does not always translate to clinical efficacy,[31] because the drug needs to be in a suitable form (ie, unionised rather than ionised form) to penetrate across cell walls to kill the bacteria—and this is directly affected by the environmental pH. Our trial will provide the first comprehensive pH data for the mouth and effects on drug PK. Lower pHs have been shown to increase the MIC for some drugs used to treat STIs.[32] In the first and only rectal azithromycin PK study, we also found that raising the gut pH by taking an acid lowering drug (esomeprazole) was associated with at least a 10-fold higher azithromycin tissue concentrations compared with those not taking this drug.[33] This is a highly relevant finding, as a previous study suggested higher azithromycin concentrations may be needed in anorectal tissue, as there was a fourfold higher MIC for *Chlamydia trachomatis* in anorectal compared with vaginal tissue.[34] Similarly, the MICs of azithromycin and ceftriaxone in NG isolates cultured from oropharynx were 1.6–1.8 times higher than in the NG isolates obtained from the urogenital tract.[35]

Applying PK data to predict an antibiotic's effectiveness, that is, its PD, varies between different classes of antibiotics and remains unclear at the oropharyngeal site. For some, the *f*T>MIC is considered to be more important (eg, for beta-lactams including cephalosporins), while for other antibiotics (eg, macrolides) the overall drug exposure (AUC) relative to MIC (*f*AUC/MIC ratio) is considered more predictive.[36] One recommendation about using PK/PD indices for predicting outcome has been published from the US Centers for Disease Control and Prevention who states that for effective NG treatment, the serum concentration should be at least 4x the MIC, for at least 10 hours after reaching its peak concentration.[37] However, this is based on data from 1964 using penicillin to treat urethral NG[38] and is therefore of limited applicability to non-penicillin treatments or infections at non-urogenital sites. For the oral space, available PK data are limited to small studies in tonsils and saliva. In addition to saliva, drug concentrations in GCF may play a role in efficacy. GCF plays a role in the progression of inflammatory oral diseases[39] which may impact oral infections and antibiotics such as azithromycin have been shown to reduce GCF volume.[40] Limited PK data in the oropharynx or oral cavity has major limitations, since we do not yet know where NG replicates in the oral space and therefore where antibiotics need to be delivered to kill NG. Therefore, understanding if an antibiotic distributes widely in oral tissue is critical.

This trial does have some limitations that must be considered when interpreting the results. Our sample is limited to males with transgender and females excluded. In addition, because of trial logistics, we had to exclude those with oropharyngeal gonorrhoea and because of this, we are unable to generate PD data as there are no bacterial outcomes in the volunteers, but we will estimate PK/PD target achievement based on the PK data and models using NG MICs. In addition, we do not have true tissue samples (eg, from biopsies) but rather swabs of surface mucosa, but this will still allow examination of drug distribution by oral cell type, for an infection that is primarily at the epithelial surface.

In conclusion, comprehensive PK data on treatments to cure oropharyngeal NG are essential if we are to maintain their effectiveness through drug optimisation when few new drugs will reach the market in the near future. Equally, methods to collect and analyse antibiotic concentrations in oral mucosal surfaces, tissue and fluids are essential to be able to apply these methods to emerging treatment in pre-marketing trials to ensure drugs in the pipeline will be effective at both oropharyngeal and anogenital sites.

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Contributors FYSK conceived and designed the original protocol with inputs from MU and JSH. MU and DAW revised the section on the microbiological and microbiota methods. NL and SHL revised the section on the recruitment and data collection. JAR, SLP and CL revised the pharmacokinetic analysis section. SCW revised the laboratory analysis section. TY revised the specimen collection section for oral specimens and fluids. CF, EPFC and DL revised the section on anorectal sampling. MH led the design of the data collection tools. FYSK and JSH wrote the first draft of the study protocol with all authors contributing to subsequent revisions and approved the protocol prior to submission.

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REFERENCES

1 World Health Organisation. actions for impact. In: *Global progress report on HIV, viral hepatitis and sexually transmitted infections, 2021. accountability for the global health sector strategies 2016–2021*, 2021.
2 National Notifiable Diseases Surveillance System. *Number of notifications of gonococcal infections, Australia, by age group and sex*, 2021.
3 Centers for Disease Control and Prevention. *Sexually Transmitted Disease Surveillance 2017 report. In: Atlanta: U.S : Department of Health and Human Services*, 2017.

4 Public Health England (PHE). *Sexually transmitted infections and screening for Chlamydia in England, 2018*. 13, 2019.
5 Chan PA, Robinette A, Montgomery M, *et al*. Extragenital infections caused by Chlamydia trachomatis and Neisseria gonorrhoeae: a review of the literature. *Infect Dis Obstet Gynecol* 2016;2016:1–17.
6 Moran JS. Treating uncomplicated Neisseria gonorrhoeae infections: is the anatomic site of infection important? *Sex Transm Dis* 1995;22:39–47.
7 Fairley CK, Cornelisse VJ, Hocking JS, *et al*. Models of gonorrhoea transmission from the mouth and saliva. *Lancet Infect Dis* 2019;19:e360–6.
8 Unemo M, Shafer WM. Antimicrobial resistance in Neisseria gonorrhoeae in the 21st century: past, evolution, and future. *Clin Microbiol Rev* 2014;27:587–613.
9 Unemo M, Lahra MM, Escher M, *et al*. Who global antimicrobial resistance surveillance for Neisseria gonorrhoeae 2017-18: a retrospective observational study. *Lancet Microbe* 2021;2:e627–36.
10 World Health Organisation. *Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics*. Geneva, Switzerland: WHO, 2017.
11 Kong FYS, Horner P, Unemo M, *et al*. Pharmacokinetic considerations regarding the treatment of bacterial sexually transmitted infections with azithromycin: a review. *J Antimicrob Chemother* 2019;74:1157–66.
12 Alirol E, Wi TE, Bala M, *et al*. Multidrug-Resistant gonorrhea: a research and development roadmap to discover new medicines. *PLoS Med* 2017;14:e1002366.
13 Kong FYS, Hatzis CL, Lau A, *et al*. Treatment efficacy for pharyngeal Neisseria gonorrhoeae: a systematic review and meta-analysis of randomized controlled trials. *J Antimicrob Chemother* 2020;75:3109–19.
14 Fifer H, Saunders J, Soni S, *et al*. 2018 UK national guideline for the management of infection with *Neisseria gonorrhoeae*. *Int J STD AIDS* 2020;31:4–15.
15 Australian Sexual Health Alliance. *Australian STI management guidelines for use in primary care*, 2021.
16 Workowski KA, Bolan GA, Centers for Disease Control and Prevention. Sexually transmitted diseases treatment guidelines, 2015. *MMWR Recomm Rep* 2015;64:1–138.
17 World Health Organisation. *Who guidelines for the treatment of Neisseria gonorrhoeae*, 2016.
18 Unemo M, Ross J, Serwin AB, *et al*. 2020 European guideline for the diagnosis and treatment of gonorrhoea in adults. *Int J STD AIDS* 20 20;2020:956462420949126:095646242094912.
19 Naicker S, Guerra Valero YC, Ordenez Meija JL, *et al*. A UHPLC-MS/MS method for the simultaneous determination of piperacillin and tazobactam in plasma (total and unbound), urine and renal replacement therapy effluent. *J Pharm Biomed Anal* 2018;148:324–33.
20 Yosipovitch G, Kaplan I, Calderon S, *et al*. Distribution of mucosal pH on the bucca, tongue, lips and palate. A study in healthy volunteers and patients with lichen planus, Behçet's disease and burning mouth syndrome. *Acta Derm Venereol* 2001;81:178–80.
21 Barbee LA, Nayak SU, Blumer JL, *et al*. A phase 1 pharmacokinetic and safety study of Extended-Duration, high-dose cefixime for cephalosporin-resistant Neisseria gonorrhoeae in the pharynx. *Sex Transm Dis* 2018;45:677–83.
22 Cao Y, Jusko WJ. Applications of minimal physiologically-based pharmacokinetic models. *J Pharmacokinet Pharmacodyn* 2012;39:711–23.
23 Landersdorfer CB, Bulitta JB, Kinzig M, *et al*. Penetration of antibacterials into bone: pharmacokinetic, pharmacodynamic and bioanalytical considerations. *Clin Pharmacokinet* 2009;48:89–124.
24 Klünemann M, Andrejev S, Blasche S, *et al*. Bioaccumulation of therapeutic drugs by human gut bacteria. *Nature* 2021;597:533–8.
25 Plummer EL, Maddaford K, Murray GL, *et al*. The impact of mouthwash on the oropharyngeal microbiota of men who have sex with men: a substudy of the omega trial. *Microbiol Spectr* 2022;10:e01757–21.
26 Jacobsson S, Golparian D, Oxelbark J, *et al*. Pharmacodynamic Evaluation of Dosing, Bacterial Kill, and Resistance Suppression for Zoliflodacin Against *Neisseria gonorrhoeae* in a Dynamic Hollow Fiber Infection Model. *Front Pharmacol* 2021;12:682135.
27 Jacobsson S, Golparian D, Oxelbark J, *et al*. Pharmacodynamic Evaluation of Zoliflodacin Treatment of *Neisseria gonorrhoeae* Strains With Amino Acid Substitutions in the Zoliflodacin Target GyrB Using a Dynamic Hollow Fiber Infection Model. *Front Pharmacol* 2022;13:874176.
28 Chow EPF, Cornelisse VJ, Read TRH, *et al*. Saliva use as a lubricant for anal sex is a risk factor for rectal gonorrhoea among men who have sex with men, a new public health message: a cross-sectional survey. *Sex Transm Infect* 2016;92:532–6.
29 Lau A, Kong FYS, Huston W, *et al*. Factors associated with anorectal *Chlamydia trachomatis* or *Neisseria gonorrhoeae* test positivity in women: a systematic review and meta-analysis. *Sex Transm Infect* 2019;95:361–7.
30 World Health Organisation. *Antimicrobial resistance: global report on surveillance*, 2014.
31 Mouton JW, Theuretzbacher U, Craig WA, *et al*. Tissue concentrations: do we ever learn? *J Antimicrob Chemother* 2008;61:235–7.
32 Barcia-Macay M, Seral C, Mingeot-Leclercq M-P, *et al*. Pharmacodynamic evaluation of the intracellular activities of antibiotics against Staphylococcus aureus in a model of THP-1 macrophages. *Antimicrob Agents Chemother* 2006;50:841–51.
33 Kong FYS, Rupasinghe TW, Simpson JA, *et al*. Pharmacokinetics of a single 1G dose of azithromycin in rectal tissue in men. *PLoS One* 2017;12:e0174372.
34 Foschi C, Salvo M, Cevenini R, *et al*. Chlamydia trachomatis antimicrobial susceptibility in colorectal and endocervical cells. *J Antimicrob Chemother* 2018;73:409–13.
35 Cole MJ, Tan W, Fifer H. Gentamicin, azithromycin and ceftriaxone in the treatment of gonorrhoea: the relationship between antibiotic MIC and clinical outcome. *J Antimicrob Chemother* 2019;75:449–57.
36 Ambrose PG, Bhavnani SM, Rubino CM, *et al*. Pharmacokinetics-Pharmacodynamics of antimicrobial therapy: it's not just for mice anymore. *Clin Infect Dis* 2007;44:79–86.
37 Newman LM, Moran JS, Workowski KA. Update on the management of gonorrhea in adults in the United States. *Clin Infect Dis* 2007;44 Suppl 3:S84–101.
38 Jaffe HW, Schroeter AL, Reynolds GH, *et al*. Pharmacokinetic determinants of penicillin cure of gonococcal urethritis. *Antimicrob Agents Chemother* 1979;15:587–91.
39 Subbarao KC, Nattuthurai GS, Sundararajan SK, *et al*. Gingival crevicular fluid: an overview. *J Pharm Bioallied Sci* 2019;11:135–9.
40 Ho W, Eubank T, Leblebicioglu B, *et al*. Azithromycin decreases crevicular fluid volume and mediator content. *J Dent Res* 2010;89:831–5.