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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 to 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 1g, cefixime 400mg or ceftriaxone 500mg plus 2g azithromycin. Participants will provide samples at 4-7 time points post-dose (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 day 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: Australian New Zealand Clinical Trial Registry - ACTRN12621000339853

Key words: Neisseria gonorrhoeae, pharmacokinetics, oropharyngeal, efficacy

Strengths and limitations of this study

- This is the first comprehensive study to collect pharmacokinetic data of drugs used to treat gonorrhoea in the oral space from four oral sites, two oral fluids and blood. The data is 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 (e.g. 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 is no data on bacterial minimum inhibitory concentrations to assess antimicrobial resistance and unable to generate pharmacodynamic data as there are no bacterial outcomes in the volunteers.

INTRODUCTION

Neisseria gonorrhoeae (NG) is the second most common bacterial sexually transmitted infection (STI) globally.[1] Over the last ten 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 (MSM) attending clinical services, respectively. Oropharyngeal infections are important because (i) cure rates at the oral site are up to 20% lower than at the genital site;[6] (ii) play a major role in transmission in the population through oral sex and use of saliva[7] and (iii) 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 World Health Organization (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 randomized controlled clinical 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, i.e. 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 1g, cefixime 400mg and ceftriaxone 500mg plus 2g 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 to 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 – collectively referred to as "oral" in this protocol. Our secondary aims are to (a) determine pharmacodynamic (PD) targets at the oral site; (b) measure pH in the oral site; (c) assess the impact of the treatments on the oral microbiome; and (d) 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 1g,[14]

ceftriaxone 500mg plus 2g azithromycin[15] and cefixime 400mg.[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_{0-24}; total: $AUC_{0-\infty}$), 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 gingival crevicular fluid (GCF).

Secondary outcomes

The magnitude of the PK/PD targets will be estimated by calculating (a) the percentage of time during which the protein unbound drug concentration exceeds the minimum inhibitory concentration (MIC) (%*f*T>MIC) for cephalosporins (b) the ratio of the area under the unbound drug concentration-time curve to the MIC (*f*AUC/MIC) for azithromycin and (c) 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 1g or cefixime 400mg, the study requires three in-person visits (over 3 days) and for those receiving dual therapy with ceftriaxone 500mg plus 2g 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 3 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/mm3 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 i.e. (a) ceftriaxone 1g[14] (Ceftriaxone-AFT, China) reconstituted in 1% lignocaine (Pfizer, Australia) as a single dose by intramuscular injection (b) ceftriaxone 500mg reconstituted in 1% lignocaine as a single dose by intramuscular injection plus 2g oral azithromycin (1g followed by 1g 6-12 hours later)[18] (Sandoz, Australia) or (c) oral cefixime 400mg[17] as single dose (Devar, Spain).

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 1g.

Reimbursement

Each participant will be reimbursed a maximum of AUD1000 for reasonable time and expenses (food and transport) - AUD500 at the conclusion of the baseline visit and a further AUD500 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 (552c; Copan, France), (b) from the posterior pharyngeal wall by swiping the site six times with FloqSwab, and (c) 15 swipes of (i) the buccal mucosa of each cheek and (ii) lateral sides of tongue using a dermal curette (4mm; Kai Medical, Japan).

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 minutes prior to the collection of saliva and GCF. 1mL 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 one minute.

Blood collection for PK and PD analysis: (a) 5ml of blood will be collected via venepuncture and plasma obtained by centrifugation at 3500rpm (2500 x g) for 15min (BD Vacutainer 102IU

lithium heparin, ref. 367885), (b) 10µL of finger prick blood will be collected using volumetric absorptive microsampling (VAMS; Neoteryx Mitra) in duplicate, (c) 10mL of whole blood to measure baseline blood biochemistry for analysis of renal and liver function (BD Vacutainer 171IU lithium heparin, ref. 367375) and haematocrit (BD Vacutainer 5.4mg 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, it's 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 5cm into anorectum and rotating gently for 5 seconds.

Collected samples and pH measurements will be taken before (baseline), 2, 4, 6, 24 and 48 hours after the antibiotic dose. For the ceftriaxone 500mg plus 2g azithromycin arm, the first post-dose sample will be taken after the ceftriaxone and first 1g dose of azithromycin. For ceftriaxone and azithromycin dual therapy, additional samples will be taken at day 7 and 14 days post-dose 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**	**0h (Baseline, before dose)**	**1-2h**	**4h**	**6h**	**d1**	**d2**	**d3-5**	**d7***	**d14***
	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 (4 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

GCF= gingival crevicular fluid; VAMS=volumetric absorptive microsampling; LFT=liver function test
*day 7 and 14 for ceftriaxone 500mg plus 2g azithromycin arm only

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 post-dose 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 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 2mL tubes containing 0.5-1mL 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 2mL 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 minutes 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 Hanna flat head meter (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 minutes, saliva will be collected into a cup over 1 minute 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]

Pharmacokinetic analysis

Nonlinear 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-, two- and three-compartment models will be evaluated, with linear, saturable or mixed-order elimination. To describe absorption, first- and zero-order, simultaneous or sequential first- 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] Inter-individual variability for the population PK parameters will be estimated where possible. Individual (posthoc) PK parameter estimates will be graphed against biological subject characteristics (e.g. 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_{0-24}, $AUC_{0-\infty}$) 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 2.0mg/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, CA, 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 (Version QIIMEq2 1.9.1). DADA2 v1.16.0 will be used to quality-filter the sequence data, infer amplicon sequence variants (ASVs), and remove chimeras. 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 at weeks 0, 1 and 2 by principal component analysis (PCA) of center-log ratio-transformed ASV level sequence data, using mixOmics (v6.12.1). 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 v2.5-7. 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.

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-00571-1 V2) 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.

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 maximize cure, minimize 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 (e.g.: 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 maximize 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 (i.e. unionized rather than ionized 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 4-fold higher MIC for *Chlamydia trachomatis* in anorectal compared to 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 i.e. 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 (e.g. for beta-lactams including cephalosporins), while for other antibiotics (e.g. 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. Additionally, 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. Additionally, we do not have true tissue samples (e.g. from 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.

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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Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet	2,11

		registered, name of intended registry	
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	#3	Date and version identifier	NA – only 1 version published
Funding	#4	Sources and types of financial, material, and other support	12
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1,12
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	12
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data	NA – no steering committees etc

		management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4,5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	6
Objectives	#7	Specific objectives or hypotheses	4,5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community	5

		clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	5,6
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	NA – participants are given single dose of drug so cannot be changed once given.
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	NA – single dose treatments
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	5,6
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg,	5

		change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	5,8
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	5,6
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce	NA – no randomization or blinding

		predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	NA
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	NA
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	NA
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and			

analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	5,6,7,8
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	NA
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	6,7,8
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	9,10

Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	9,10
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	9,10
Methods:			
Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	NA – no DMC
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	NA- no interim analyses
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	8

Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NA
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	10
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	NA
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	5,6
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	8
Declaration of	#28	Financial and other competing interests for	11

interests		principal investigators for the overall trial and each study site	
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	11
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	11
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	12
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	11
Appendices			
Informed consent	#32	Model consent form and other related	Supp

materials		documentation given to participants and authorised surrogates	
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	6.7,8,9

BMJ Open

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

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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 to 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 1g, cefixime 400mg or ceftriaxone 500mg plus 2g azithromycin. Participants will provide samples at 4-7 time points post-dose (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 day 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: Australian New Zealand Clinical Trial Registry - ACTRN12621000339853

Key words: Neisseria gonorrhoeae, pharmacokinetics, oropharyngeal, efficacy

Strengths and limitations of this study

- This is the first comprehensive study to collect pharmacokinetic data of drugs used to treat gonorrhoea in the oral space from four oral sites, two oral fluids and blood. The data is 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 (e.g. 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 is no data on bacterial minimum inhibitory concentrations to assess antimicrobial resistance and unable to generate real-world pharmacodynamic 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 ten 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 (MSM) attending clinical services, respectively. Oropharyngeal infections are important because (i) cure rates at the oral site are up to 20% lower than at the genital site;[6] (ii) play a major role in transmission in the population through oral sex and use of saliva[7] and (iii) 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 World Health Organization (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 randomized controlled clinical 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, i.e. 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 1g, cefixime 400mg and ceftriaxone 500mg plus 2g 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 to 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 – collectively referred to as "oral" in this protocol. Our secondary aims are to (a) determine pharmacodynamic (PD) targets at the oral site; (b) measure pH in the oral site; (c) assess the impact of the treatments on the oral microbiome; and (d) 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 1g,[14]

ceftriaxone 500mg plus 2g azithromycin[15] and cefixime 400mg.[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_{0-24}; total: $AUC_{0-\infty}$), 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 gingival crevicular fluid (GCF).

Secondary outcomes

The magnitude of the PK/PD targets will be estimated by calculating (a) the percentage of time during which the protein unbound drug concentration exceeds the minimum inhibitory concentration (MIC) (%*f*T>MIC) for cephalosporins (b) the ratio of the area under the unbound drug concentration-time curve to the MIC (*f*AUC/MIC) for azithromycin and (c) 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 1g or cefixime 400mg, the study requires three in-person visits (over 3 days) and for those receiving dual therapy with ceftriaxone 500mg plus 2g 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 3 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/mm3 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 i.e. (a) ceftriaxone 1g[14] (Ceftriaxone-AFT, China) reconstituted in 1% lignocaine (Pfizer, Australia) as a single dose by intramuscular injection (b) ceftriaxone 500mg reconstituted in 1% lignocaine as a single dose by intramuscular injection plus 2g oral azithromycin tablet (1g followed by 1g 6-12 hours later)[18] (Sandoz, Australia) or (c) oral cefixime 400mg[17] capsule as single dose (Denvar, Spain). The second 1g 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 1g.

Reimbursement

Each participant will be reimbursed a maximum of AUD1000 for reasonable time and expenses (food and transport) - AUD500 at the conclusion of the baseline visit and a further AUD500 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 (552c; Copan, France), (b) from the posterior pharyngeal wall by swiping the site six times with FloqSwab, and (c) 15 swipes of (i) the buccal mucosa of each cheek and (ii) lateral sides of tongue using a dermal curette (4mm; 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 minutes prior to the

collection of saliva and GCF. 1mL 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 one minute.

Blood collection for PK and PD analysis: (a) 5ml of blood will be collected via venepuncture and plasma obtained by centrifugation at 3500rpm (2500 x g) for 15min (BD Vacutainer 102IU lithium heparin, ref. 367885), (b) 10µL of finger prick blood will be collected using volumetric absorptive microsampling (VAMS; Neoteryx Mitra) in duplicate, (c) 10mL of whole blood to measure baseline blood biochemistry for analysis of renal and liver function (BD Vacutainer 171IU lithium heparin, ref. 367375) and haematocrit (BD Vacutainer 5.4mg 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, it's 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 5cm into anorectum and rotating gently for 5 seconds.

Collected samples and pH measurements will be taken before (baseline), 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 500mg plus 2g azithromycin arm, the first post-dose sample will be taken after the ceftriaxone and first 1g dose of azithromycin. For ceftriaxone and azithromycin dual therapy, additional samples will be taken at day 7 and 14 days post-dose 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**	**0h* (Baseline, before dose)**	**1-2h***	**4h***	**6h***	**d1**	**d2**	**d3-5**	**d7****	**d14****
	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 (4 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

GCF= gingival crevicular fluid; VAMS=volumetric absorptive microsampling; LFT=liver function test
*Samples taken at times 0-6h are all taken during the same visit i.e. during the 'day stay'
**day 7 and 14 for ceftriaxone 500mg plus 2g azithromycin arm only

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 post-dose 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 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 2mL tubes containing 0.5-1mL 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 2mL 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 minutes 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 Hanna flat head meter (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 minutes, saliva will be collected into a cup over 1 minute 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]

Pharmacokinetic analysis

Nonlinear 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-, two- and three-compartment models will be evaluated, with linear, saturable or mixed-order elimination. To describe absorption, first- and zero-order, simultaneous or sequential first- 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] Inter-individual variability for the population PK parameters will be estimated where possible. Individual (posthoc) PK parameter estimates will be graphed against biological subject characteristics (e.g. 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_{0-24}, $AUC_{0-\infty}$) 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 2.0mg/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, CA, 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 (Version QIIMEq2 1.9.1). DADA2 v1.16.0 will be used to quality-filter the sequence data, infer amplicon sequence variants (ASVs), and remove chimeras. 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 at weeks 0, 1 and 2 by principal component analysis (PCA) of center-log ratio-transformed ASV level sequence data, using mixOmics (v6.12.1). 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 v2.5-7. 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-00571-1 V2) 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.

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 maximize cure, minimize 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 (e.g.: 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 maximize 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 (i.e. unionized rather than ionized 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 4-fold higher MIC for *Chlamydia trachomatis* in anorectal compared to 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 i.e. 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 (e.g. for beta-lactams including cephalosporins), while for other antibiotics (e.g. 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. Additionally, 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. Additionally, we do not have true tissue samples (e.g. from 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.

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: FSYK 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 CBL revised the pharmacokinetic analysis section. SCW revised the laboratory analysis section. TY revised the specimen collection section for oral specimens and fluids. CKF, EPFC and DAL revised the section on anorectal sampling. MAH 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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Patient consent for publication: Not applicable.

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Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet	2,11

		registered, name of intended registry	
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	#3	Date and version identifier	NA – only 1 version published
Funding	#4	Sources and types of financial, material, and other support	12
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	1,12
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	12
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data	NA – no steering committees etc

		management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4,5
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	6
Objectives	#7	Specific objectives or hypotheses	4,5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community	5

		clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	5,6
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	6
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	NA – participants are given single dose of drug so cannot be changed once given.
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	NA – single dose treatments
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	5,6
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg,	5

		change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	5,8
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	9
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	5,6
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce	NA – no randomization or blinding

		predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	NA
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	NA
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	NA
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and			

analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	5,6,7,8
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	NA
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	6,7,8
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	9,10

Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	9,10
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	9,10
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	NA – no DMC
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	NA- no interim analyses
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	8

Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NA
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	10
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	NA
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	5,6
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	8
Declaration of	#28	Financial and other competing interests for	11

interests		principal investigators for the overall trial and each study site	
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	11
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	11
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	12
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	11
Appendices			
Informed consent	#32	Model consent form and other related	Supp

materials		documentation given to participants and authorised surrogates	
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	6.7,8,9