Clinical Microbiology and Infection 26 (2020) 1630–1635

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Narrative review

Pharmacokinetic/pharmacodynamic considerations for new and current therapeutic drugs for uncomplicated gonorrhoea—challenges and opportunities

Ursula Theuretzbacher [1, *], Lindley Barbee [2], Kristie Connolly [3], George Drusano [4], Prabha Fernandes [5], Edward Hook [6], Ann Jerse [3], John O'Donnell [7], Magnus Unemo [8], Françoise Van Bambeke [9], Brian VanScoy [10], Peter Warn [11], Brian J. Werth [12], François Franceschi [13], Emilie Alirol [13]

[1] *Centre for Anti-Infective Agents, Vienna, Austria*
[2] *University of Washington, Division of Allergy and Infectious Diseases, Department of Medicine, Seattle, WA, USA*
[3] *Uniformed Services University, Bethesda, MD, USA*
[4] *Institute for Therapeutic Innovation, University of Florida, Lake Nona, FL, USA*
[5] *Chair, Scientific Advisory Committee, GARDP, Geneva, Switzerland*
[6] *University of Alabama at Birmingham, Birmingham, AL, USA*
[7] *Entasis Therapeutics, Waltham, MA, USA*
[8] *WHO Collaborating Centre for Gonorrhoea and other Sexually Transmitted Infections, Örebro University, Örebro, Sweden*
[9] *Université Catholique de Louvain, Brussels, Belgium*
[10] *Institute for Clinical Pharmacodynamics, Latham, New York, USA*
[11] *Magic Bullet Consulting, London, UK*
[12] *University of Washington, School of Pharmacy, Seattle, WA, USA*
[13] *Global Antibiotic R&D Partnership (GARDP), Geneva, Switzerland*

ARTICLE INFO

Article history:
Received 13 May 2020
Received in revised form
10 July 2020
Accepted 6 August 2020
Available online 13 August 2020

Editor: W. Couet

Keywords:
Gonorrhoea
Neisseria gonorrhoeae
PK/PD models
Preclinical development
Sexually transmitted disease
Treatment

ABSTRACT

Background: Increasing multidrug resistance rates in *Neisseria gonorrhoeae* have raised concerns and an urgent call for new antibiotics for treatment of gonorrhoea. Several decades of subdued drug development in this field and the recent failures of two new antibiotics to show non-inferiority compared with the current first-line antibiotics ceftriaxone plus azithromycin highlight the need for improved preclinical tools to predict clinical outcome of new drugs in the development process.
Objectives: To summarize current pharmacokinetic/pharmacodynamic (PK/PD) knowledge and dose-finding strategies for antibiotics against gonorrhoea.
Sources: Literature review of published papers and discussions by global experts at a special workshop on this topic.
Content: We review current knowledge of gonococcal specific PK/PD principles and provide an update on new *in vitro* and *in vivo* models to correlate drug exposure with clinical outcome, and identify challenges and gaps in gonococcal therapeutic research.
Implications: Identifying the ideal antimicrobial agent and dose for treating uncomplicated urogenital and pharyngeal gonococcal disease requires appropriate validated non-clinical PK/PD models. Recent advances in adapting *in vitro* and *in vivo* models for use in gonorrhoea are an important step for enabling the development of new drugs with reduced risk of failure in Phase 3 clinical development and diminish the risk of emergence of resistance. **Ursula Theuretzbacher, Clin Microbiol Infect 2020;26:1630**
© 2020 Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases.

* Corresponding author. U. Theuretzbacher, Centre for Anti-Infective Agents, Vienna, Austria.
E-mail address: utheuretzbacher@cefaia.com (U. Theuretzbacher).

https://doi.org/10.1016/j.cmi.2020.08.006
1198-743X/© 2020 Published by Elsevier Ltd on behalf of European Society of Clinical Microbiology and Infectious Diseases.

Introduction

Gonorrhoea, caused by *Neisseria gonorrhoeae*, is among the most common sexually transmitted infections with an estimated global incidence of 87 million cases in 2016 [1–3]. Reports increasingly highlight progressive resistance of *N. gonorrhoeae* to the antibiotics most commonly used for treatment. In many countries, resistance to ciprofloxacin is exceedingly high, azithromycin resistance is present, and decreased susceptibility or resistance to ceftriaxone has emerged [4,5]. Though azithromycin resistance occurs globally, resistance to recommended dual azithromycin and ceftriaxone therapy is rare and for the moment this combination can be used with confidence [3,4,6]. However, for persons who cannot receive these drugs, there are no alternatives and no sustainable therapeutic option for extensively drug resistant *N. gonorrhoeae* is currently available [7]. As a result, and in response to prioritization by the WHO [8], companies and investors have expressed interest in developing new treatments or including this indication in their portfolio as reflected in the preclinical and clinical antibacterial pipeline review [9,10]. Unfortunately, however, two new oral antibiotics evaluated in Phase 3 randomized clinical controlled trials (RCTs) failed to show non-inferiority as single-dose monotherapies [11–13]. Oral solithromycin was compared with the currently recommended single-dose combination of intramuscular ceftriaxone plus azithromycin and oral delafloxacin with intramuscular ceftriaxone administered as single-dose therapies. These failed Phase 3 RCTs and limited experience in developing new drugs for uncomplicated urogenital gonorrhoea raised concern in the scientific and drug development communities [11–13]. A combination of ceftriaxone plus azithromycin, which has been the standard of care in the USA for three decades, has been required by the US Food and Drug Administration (FDA) as the comparator regimen for ongoing trials, raising further the bar for new drugs that are evaluated as monotherapy [14–18]. Currently regulators require that the new drug be administered as a single dose, in line with clinical practice, and that the lower bound of the non-inferiority margin confidence interval be above 10%. As ceftriaxone plus azithromycin has demonstrated efficacy of 99%–100% in individuals infected with susceptible *N. gonorrhoeae* in clinical trials, the new product administered in monotherapy must have ≥95% efficacy. Recently, the FDA has allowed gepotidacin to be administered as a two-dose regimen in a Phase 3 gonorrhoea RCT (ClinicalTrials.gov Identifier: NCT04010539). This more flexible dosing schedule may facilitate development of new treatment options for resistant *N. gonorrhoeae* that would not be suitable for a single dose therapy. Common pharmacokinetic/pharmacodynamic (PK/PD) models for optimizing dosage and correlating exposure with clinical outcome have not been rigorously applied to gonorrhoea. These models may require modification and validation but could support greatly the development of new gonorrhoea treatments.

In November 2019, Global Antibiotic Research and Development Partnership (GARDP) organized an expert workshop to discuss the current state of research, existing gaps, challenges and opportunities of *in vitro* and *in vivo* models that link contemporary PK and PD science to prediction of dosage regimens for ideal clinical and microbiological eradication of infection and suppression of resistance emergence. This review focuses on the recent scientific progress in adapting non-clinical models to accommodate research for new drugs against *N. gonorrhoeae*, current knowledge gaps and translational challenges.

Special considerations for *Neisseria gonorrhoeae*

Site of infection

Neisseria gonorrhoeae primarily invades and colonizes urogenital epithelial cells, e.g. cervix, Fallopian tubes and the urethra, both male and female [19,20]. Anorectal and pharyngeal infections are also relatively common and may warrant different considerations when compared with urogenital sites. Within epithelial cells a subpopulation of intracellular gonococci is able to survive by inhibiting autophagosome maturation and lysosome fusion [21,22]. Survival of intracellular gonococci present in vacuoles or free in epithelial cytoplasm seems transitory and is followed by death of the organisms or exocytosis. Although the intracellular site is well recognized, the extent to which it is relevant for treatment outcomes is unknown. Furthermore, *N. gonorrhoeae* is well adapted to its obligate human host and manipulates neutrophil functionality and expresses defensins against neutrophil antimicrobial activities [23]. The bacteria attach to polymorphonuclear neutrophils (PMNs), then survive and replicate inside the PMNs by residing in immature phagosomes [24–27]. Although serum levels have been long used to assess antibiotic exposure for gonorrhoea therapy, only in very rare cases, such as disseminated gonococcal infection, is blood a relevant pharmacological site [28]. Based on the current evidence, potential *N. gonorrhoeae* infection sites include extracellular (interstitial fluids of cervical, urethral, rectal and pharyngeal tissues, and in vaginal fluids, urine, saliva), as well as intracellular sites (epithelial cells and PMNs). The relevance of each of these potential pharmacological compartments as important determinants of treatment outcome and PK input for PK/PD models is still debated.

Antibiotic concentrations at potential sites of infection

Physicochemical properties (e.g. hydrophilicity and lipophilicity) and other factors such as protein binding determine antibiotic distribution in the body, but antibiotic concentrations at potential infection sites of gonorrhoea are poorly defined or unknown. Plasma antibiotic concentrations are easily measured and are widely available but may not reflect active concentrations at distant sites. For hydrophilic antibiotics (such as β-lactam antibiotics), which distribute by passive diffusion, the concentrations in plasma and interstitial fluids of tissues should equilibrate at steady state. In such cases plasma exposures could reflect extracellular compartments of most tissues but local variation in pH, protein binding, cell junctions/inflammation and clearance may alter local pharmacodynamics [29]. For other antibiotic classes, however, this assumption does not apply; an example being azithromycin, which achieves intracellular concentrations that are several-fold higher than its very low plasma and extracellular fluid concentrations [30,31].

The relevance of the intracellular localization of *N. gonorrhoeae* for predictive PK/PD studies is still debated, but *ex vivo* models exist to assess intracellular antibiotic penetration in relation to blood levels and intracellular activity. These include human primary urethral cell lines, human endometrial cell lines, human monocytic cell lines and human PMNs [20,32,33]. Aminoglycosides only slowly penetrate into host cells; for this reason, gentamicin is often used to kill extracellular *N. gonorrhoeae* in *in vitro* models, leaving just intracellular bacteria [34]. In contrast, β-lactams readily diffuse through cellular membranes but do not accumulate within the cell (extracellular: intracellular ratio ~1), and macrolides accumulate in

acidic compartments and are substrates for efflux (P-glycoprotein) [35,36]. Azithromycin is also characterized by non-saturable uptake into phagocytic cells, mainly lysosomes, with a high degree of retention [37,38]. Fluoroquinolones diffuse and accumulate in cells to variable levels and may redistribute in compartments while gepotidacin diffuses and accumulates only minimally (extracellular: intracellular ratio ~1.6), partly in lysosomes and partly in the cytosol [39,40]. As antibiotics may reach different concentrations in different subcellular compartments, there is no direct correlation between the global intracellular accumulation of antibiotics and the level of their intracellular activity. Similarly, bacteria reside in separate intracellular compartments that may not be equally reached by antibiotics [41]. There is little information available about the intracellular compartmental concentrations because total intracellular concentrations are derived from lysed cells and mix different intracellular compartments. Such measured total intracellular concentrations might therefore not predict intracellular activity and robust basic data are still missing [42].

Pharyngeal gonorrhoea is more difficult to treat than urogenital infection and this is a common site of treatment failures for all drugs evaluated to date [43–45]. Ineffective treatment makes the pharynx a potential reservoir for transmission and the emergence of antibacterial resistance. Further, it has been suggested that resistance may arise more readily at the pharynx because of the potential transfer of resistance genes from commensal pharyngeal *Neisseria* to gonococci [46,47]. Discussions of the magnitude of the role of the oropharynx as a potential site of gonorrhoea transmission are ongoing [48,49]. Bacteria in the oropharynx are mostly attached to epithelial cells but saliva and pharyngeal fluid contain cells with intracellular survival compartments. It is not known if salivary or plasma concentrations reliably reflect antimicrobial exposures at the oropharyngeal site of infection [42,50].

It is still unclear which measurement represents the appropriate concentration at the site of infection and should be used as input for *in vitro* PK/PD models. The role of plasma concentrations as a surrogate measure for extracellular concentrations or in some specific cases for intracellular concentrations depends on the physicochemical properties of the drug and needs to be determined individually.

Development of antibiotic resistance

For gonorrhoea, suppression of resistance emergence in bacterial subpopulations and its relevance to clinical outcomes is unknown. In the past, general antimicrobial exposure to drugs that are also used for the treatment of gonorrhoea has been shown to be the significant overall driver in the evolution and subsequent emergence of resistance [51]. The emergence of resistance of two *N. gonorrhoeae* strains against gepotidacin with an MIC increase of $\geq$32-fold during single-dose treatment in a Phase 2 trial raises concern for research and development programmes [51]. Rapid eradication of bacteria and suppression of resistance are different therapeutic goals [42,51]. In theory, some antibiotics (or combinations) might show less rapid kill but may instead more effectively suppress resistance emergence or vice versa. For gonorrhoea, how PK/PD data might predict the suppression of resistance development is largely unknown. Challenges to assessing the rate of gonococcal antimicrobial resistance emergence include: organism-specific factors such as fastidious growth outside the human body, genomic background, susceptibility and bacterial burden of the same strain in divergent environments and infection sites; the influence of commensal *Neisseria* species, particularly in the pharynx; drug-related factors such as single- or multiple-target drugs, protein binding, extracellular versus intracellular distribution; and patient-specific factors such as pH and iron concentrations at infection sites, the immune response at infection sites, sexual or other practices. Although single-dose treatments for gonorrhoea have been effective for decades, this therapy concept may be suboptimal for suppressing the emergence of resistance. In general, there is no information available about drug dosing to optimize killing while at the same time suppressing the emergence of resistance.

***In vitro* models**

Non-clinical PK/PD models play a critical predictive role in designing human dosage regimens and are essential tools for drug development [52,53]. Conventional static and dynamic *in vitro* models can be adapted for *N. gonorrhoeae*, including kill curve analysis for single antibiotics or antibiotic combination testing [54]. However, the general poor growth of *N. gonorrhoeae* in liquid media imposes certain limitations. In contrast to usual PK/PD indices, which are based on achieving stasis or 1 log (−2 log) reduction of the bacterial burden [52], the required reduction in PK/PD models of *N. gonorrhoeae* to achieve eradication of the bacteria in clinical studies is not known. Obtaining complete bacterial eradication with a single-dose antibiotic is a high bar.

The hollow-fibre infection model (HFIM) has become widely used for characterizing efficacy and emergence of resistance using simulated human blood or tissue PK profiles.

The usefulness of a surrogate pathogen approach using *Staphylococcus aureus* in the HFIM is controversial because it does not consider the specific infection biology of *N. gonorrhoeae* [55].

Challenges to adaptation of this model for *N. gonorrhoeae* included growing the bacteria in continuous liquid culture while reducing the high variability. Recently, the HFIM was adapted to grow *N. gonorrhoeae* over a 7-day period with minimal autolysis and was used with ceftriaxone, ciprofloxacin and gepotidacin [56]. In these experiments results correlated with animal data and clinical outcomes when applied to ceftriaxone with simulated blood concentrations following a single 250-mg intramuscular dose. The range of gepotidacin dosing regimens evaluated within the HFIM provided a full exposure response and identified exposures in blood that suppressed resistance emergence over the 7-day period. These data were used to help choose dosing regimens for the treatment of individuals with uncomplicated gonorrhoea enrolled in a pivotal Phase 3 RCT that is currently ongoing (ClinicalTrials.gov Identifier: NCT04010539). The predictive value of HFIM for *N. gonorrhoeae* infection is yet to be established. Challenges such as strain variability and influence of the culture medium need optimization. The HFIM model requires more basic research into the activity of existing antibiotics and the correlations with historic clinical outcomes to validate this system. Overall, the benefits of having a dynamic *in vitro* system that can identify PK/PD relationships as well as exposures associated with prevention of emergence of resistance could prove invaluable for the characterization of new and old antimicrobials.

Intracellular *N. gonorrhoeae* infection models have been published that use endocervical and ectocervical tissue biopsies and endocervical epithelial cell lines but they usually do not focus on intracellular activity of antibiotics [19,57,58]. In some models, intracellular bacterial counts tend to decrease over time even in the absence of antibiotics [21]. It is unknown how and to what extent *N. gonorrhoeae* moves between extracellular and intracellular spaces and if intracellular survival is transitory in epithelial cells within these models. It also seems that the intracellular fate of gonococci may vary depending on the bacterial strain and cell type used for the experiment. Based on other models of intracellular infections, particularly with staphylococci and *Listeria*, fluoroquinolones are effective in all subcellular compartments when

tested in *in vitro* systems [39,59]. β-lactams such as ceftriaxone and penicillin with low MICs may also act intracellularly, as they show high serum levels and cellular concentrations close to the extracellular ones. Macrolides are usually static despite their high intracellular accumulation levels and their activity is compromised by acidic pH in vacuolar compartments. Solithromycin is more stable in acidic pH conditions and more active in intracellular assays than azithromycin [60].

A human three-dimensional endometrial epithelial cell model using the HEC-1A cell line and the rotating wall vessel bioreactor technology has been recently developed to study host–microbe interactions *in vitro* [61]. It is not known if this technology could be adapted for studying PK/PD relationships. Several other models that are used for a variety of infections may be adapted for studying gonorrhoea. Established mucosal tissue models using colon, endometrial and uroepithelial cell lines may be suitable for further studies. *In vitro* three-dimensional cell cultures that are able to more accurately reflect the complex *in vivo* microenvironment compared with the standard *in vitro* tissue model could be adapted to gonococcal infections. Confocal imaging would allow analysis of more complicated tissue models and intracellular bacteria using high-content screening techniques.

In vivo models

In vivo models play a critical role in characterizing the PK/PD for antibacterial agents and are an integral part of dose finding or dose optimization strategies. The mouse thigh infection model has been used for studying the most relevant pathogen–antibiotic pairs and the PK/PD relationships correlate reasonably well with results of clinical studies [52]. Until recently, the strict human specificity of *N. gonorrhoeae* limited the ability of such models to mimic adaptations to human mucosa. More recently the oestradiol-preconditioned mouse model of *N. gonorrhoeae* genital tract infection has allowed prolonged *N. gonorrhoeae* mucosal infection and has proved to be a valuable model for mimicking the colonization kinetics of human cervical infections in women of reproductive age and testing of novel therapeutic and prophylactic compounds [22,62]. In this model, bacterial cells are seen within PMNs and in the lamina propria. It was also shown that PK data are predictive of *in vivo* efficacy for cefixime and ceftriaxone against susceptible and resistant *N. gonorrhoeae* strains based on the PK/PD index for β-lactams (*f*T > MIC) in this model [63]. Encouragingly, the oestradiol-preconditioned mouse model confirmed the efficacy of a single dose (5 mg/kg) of ceftriaxone against a susceptible strain and free drug blood concentrations above the MIC during 24 hours (*f*T > MIC of 24 hours). This magnitude of the PK/PD index is likely be higher compared with most other pathogens where complete eradication of the bacteria is not usually the target (usually 1–3 log reduction in bacterial load) [52]. For a ceftriaxone-resistant strain, administration of multiple doses of ceftriaxone produced an *f*T > MIC of 23 hours and were able to eradicate the bacteria in a majority (90%) of mice. These results revealed a clear relationship for ceftriaxone between MIC, plasma levels and bacterial clearance rates in the gonorrhoea mouse model. The magnitude of the index observed in mice reflected those observed in humans requiring doses that yielded an *f*T > MIC for at least 20–24 hours [63]. In contrast, there is a dose-dependent response for ciprofloxacin with an *f*AUC/MIC driver in the model, although for ciprofloxacin the correlation with clinical outcome was less clear. The oestradiol-preconditioned mouse model may be useful to establish dose–response relationships that are relevant for acute uncomplicated urogenital infections in women. Other types of infections such as urethral infection in men, pharyngeal, rectal or asymptomatic colonization and prevention of long-term complications will need appropriate pharmacokinetic data in humans and may need alternative models.

Translational considerations

Though critical gaps in our knowledge exist and point to future needs for research (Table 1) important progress has been made. The HFIM and the oestradiol-preconditioned mouse model represent important steps in establishing PK/PD relationships as the basis of optimizing the dose of existing drugs and for defining dose-finding strategies during the drug development process, but both have limitations. Furthermore, the relevance of the different infection sites including the intracellular compartments for human infections and the appropriate exposure at these sites requires clarification; in addition the validity of using simulated plasma concentrations in *in vitro* models needs to be confirmed for each antibiotic class by comparing the efficacy of established antibiotics with clinical outcome data including clinical failures to elucidate the role of plasma concentrations as surrogate for other infection sites, as input into PK/PD models. Ceftriaxone, cefixime and ciprofloxacin can serve as reference points to establish exposure–outcome relationships and calibrate new models. Additionally, different strains with a range of MICs above and below the current clinical resistant breakpoint should be tested to consider MIC creep and potentially adjust dosing regimens [64]. If plasma concentrations are shown to be predictive for other infection sites, population PK and Monte Carlo simulations to reflect the diversity inherent within patient populations need to be implemented to assist in establishing dosing regimens [64]. So far, such simulations were mainly focused on established antibiotics with known PK data, MIC values and microbiological/clinical outcome information

Table 1
Future areas for pharmacokinetics/pharmacodynamics (PK/PD) research on drugs for gonorrhoea

Site of infection	Study relevance of intracellular location and antibiotic concentrations, local factors, e.g. biofilm, protein binding, bacterial burden, clumping, influence of commensals, immune system
Relevant antibiotic concentration	Which concentrations to use for modelling: do serum concentrations reflect the concentrations required at urogenital, rectal and pharyngeal sites of infection?
PK/PD index	Adapt PK/PD indices that consider single-dose and multiple-dose regimens and the requirement for sterilization
Strain-dependent factors	Define impact of strain variability on modelling and clinical cure
HFIM	Validate and explore HFIM with old antibiotics and correlate the results with known clinical outcome, explore new knowledge such as PK input, consider strain-specific factors
In vivo models	Develop *in vivo* models for infections other than cervical gonorrhoea, expand studies and correlate the results with known clinical outcome of old antibiotics
Clinical breakpoints	Provide information to reassess clinical breakpoints, define failure thresholds
Dosing regimens	Explore different dosing regimens: single-dose, multiple-dose and combination therapy
Resistance	Integrate the goals of fast killing with minimized emergence of resistance
Research environment	Intensify international collaborative actions and research efforts

Table 2
Clinical development and regulatory challenges for new drugs against *Neisseria gonorrhoeae*

Dosing regimens	New antibiotics may not reach the optimal magnitude of their pharmacokinetics/pharmacodynamics driver with a single dose and may need new dosage regimens to be compared to the combination ceftriaxone plus azithromycin therapy
Pharyngeal gonorrhoea	Difficult to treat and usually asymptomatic, treatment failures occur
Gender considerations	Clinical presentation, inclusion criteria in clinical trials, dosing should be considered for future clinical trials
Antibiotic combinations	Antibiotic combination (ceftriaxone + azithromycin) is recommended because of chlamydial co-infections and to hypothetically mitigate emergence and/or spread of ceftriaxone resistance, which remains unproven
Co-infections	Most common: *Chlamydia trachomatis*, *Trichomonas vaginalis*, *Mycoplasma genitalium*; should be evaluated in clinical trials
Persons with human immunodeficiency virus infection	Some of the anti-human immunodeficiency virus drugs are CYP3A inhibitors or inducers and impact drug metabolism, so concentrations of the new drugs could be affected
Epidemiologically relevant subpopulations	Some subpopulations such as men who have sex with men or commercial sex workers are also at high risk for re-infection, making long-term evaluations in these communities challenging
Enrolment challenges	Clinical trial design constraints may hinder enrolment of populations at high risk for gonorrhoea such as adolescents, women (often without symptoms), and persons infected with antibiotic-resistant *N.gonorrhoeae*
Regulatory	Current regulatory standards require integration of public health issues into the clinical development pathway (single dose). Culture remains the standard for clinical trials and antimicrobial susceptibility testing despite poor sensitivity but nucleic acid amplification tests have become the preferred tests in most clinical settings

assuming the relevance of blood concentrations [65]. There is no established model to predict the most appropriate dose for minimizing the emergence of antibiotic resistance in gonorrhoea. Available data suggest a single dose cannot accomplish this goal for most antibiotics. Additionally, clinical development and regulatory challenges for new drugs against *N. gonorrhoeae* exist and are listed in Table 2.

The development of successful treatments for gonorrhoea will require further understanding of PK/PD determinants for effective therapy and prevention of emergence of resistance, validation of non-clinical models, and carefully designed clinical trials [66]. A concerted effort and collaboration of all experts will be necessary to advance our knowledge of appropriate dose-finding strategies and clinical development questions to provide the basis for a successful development of new antibiotics against drug-resistant gonorrhoea.

Transparency declaration

UT has received honoraria from the Global Antibiotic Research and Development Partnership (GARDP); EA and FF are employees of GARDP; PF is Chair of the Scientific Advisory Committee for GARDP that is developing zoliflodacin; AJ has received grants from Reoxcyn, Inc., Yaso Therapeutics, Microbiotix, Inc., Venatorx, Inc. and GlaxoSmithKline, Siena, outside the submitted work; LB has received non-financial support from Hologic, grants from SpeeDX and personal fees from Nabriva, outside the submitted work; PF is chair of the Scientific Advisory Committee of GARDP; JO is an employee of Entasis Therapeutics; FVB has received grants from Debiopharm, Merlion Pharmaceuticals, GSK, Eumedica, outside the submitted work; BW has received grants from Shionigi Inc., outside the submitted work.

Funding

The Global Antibiotic Research and Development Partnership supported the organization of the expert workshop.

Contributions

All authors have contributed to the discussions about the content of the manuscript, revising, editing and approving the final manuscript. UT wrote the first draft.

Acknowledgements

Carolyn Deal and Ann Eakin presented at the expert workshop and contributed valuable insights into new gonorrhoea research. They provided inspiring input to the discussion and to this manuscript. We thank Teodora Elvira Wi, Steven Gelone, Paul Meo, Guennaëlle Dieppois and Annick Ménétrey for their contributions to the discussion.

References

[1] Rowley J, Vander Hoorn S, Korenromp E, Low N, Unemo M, Abu-Raddad LJ, et al. Chlamydia, gonorrhoea, trichomoniasis and syphilis: global prevalence and incidence estimates, 2016. Bull World Health Organ 2019;97:548–62.
[2] Vincent LR, Jerse AE. Biological feasibility and importance of a gonorrhea vaccine for global public health. Vaccine 2019;37:7419–26.
[3] Unemo M, Seifert HS, Hook EW, Hawkes S, Ndowa F, Dillon J-AR. Gonorrhoea Nat Rev Dis Primers 2019;5:79.
[4] Unemo M, Lahra MM, Cole M, Galarza P, Ndowa F, Martin I, et al. World Health Organization Global Gonococcal Antimicrobial Surveillance Program (WHO GASP): review of new data and evidence to inform international collaborative actions and research efforts. Sex Health 2019;16:412–25.
[5] Suay-García B, Pérez-Gracia MT. Future prospects for *Neisseria gonorrhoeae* treatment. Antibiotics (Basel) 2018;7:49.
[6] Sánchez-Busó L, Harris SR. Using genomics to understand antimicrobial resistance and transmission in *Neisseria gonorrhoeae*. Microb Genom 2019;5:e000239.
[7] Alirol E, Wi TE, Bala M, Bazzo ML, Chen X-S, Deal C, et al. Multidrug-resistant gonorrhea: a research and development roadmap to discover new medicines. PLoS Med 2017;14:e1002366.
[8] Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Inf Dis 2018;18:318–27.
[9] Theuretzbacher U, Bush K, Harbarth S, Paul M, Rex JH, Tacconelli E, et al. Critical analysis of antibacterial agents in clinical development. Nat Rev Microbiol 2020;18:286–98.
[10] Theuretzbacher U, Outterson K, Engel A, Karlén A. The global preclinical antibacterial pipeline. Nat Rev Microbiol 2020;18:275–85.
[11] Fernandes P, Craft JC. Phase 3 trial of treating gonorrhoea with solithromycin. Lancet Inf Dis 2019;19:928.
[12] Hook 3rd EW, Golden MR, Taylor SN, Henry E, Tseng C, Swerdlow J, et al. Efficacy and safety of single dose oral delafloxacin compared with intramuscular ceftriaxone for uncomplicated gonorrhea treatment: an open-label, non-inferiority, Phase 3, multicenter, randomized study. Sex Transm Dis 2019. Jan 19.
[13] Chen MY, McNulty A, Avery A, Whiley D, Tabrizi SN, Hardy D, et al. Solithromycin versus ceftriaxone plus azithromycin for the treatment of uncomplicated genital gonorrhoea (SOLITAIRE-U): a randomised phase 3 non-inferiority trial. Lancet Inf Dis 2019;19:833–42.
[14] CDC. 2015 Sexually transmitted diseases treatment guidelines 2015. Available from: https://www.cdc.gov/std/tg2015/gonorrhea.htm.
[15] WHO. WHO guidelines for the treatment of *Neisseria gonorrhoeae* geneva, Switzerland. Available from: https://apps.who.int/iris/bitstream/handle/10665/246114/9789241549691-eng.pdf.
[16] FDA. Developing drugs for treatment of uncomplicated gonorrhea: guidance for Industry 2015. Available from: https://www.fda.gov/media/88904/download.

[17] EMA. EMA/844951/2018Rev. 3, Guideline on the evaluation of medicinal products indicated for treatment of bacterial infections—London, UK. Available from: https://www.ema.europa.eu/en/documents/scientific-guideline/draft-guideline-evaluation-medicinal-products-indicated-treatment-bacterial-infections-revision-3_en.pdf; 2018.

[18] Handsfield HH, Zenilman JM. Standards for treatment and control regimens in therapeutic trials for gonorrhea: lessons from a “failed” trial. Sex Transm Dis 2019;46:287–9.

[19] Edwards JL, Apicella MA. The molecular mechanisms used by *Neisseria gonorrhoeae* to initiate infection differ between men and women. Clin Microbiol Rev 2004;17:965–81.

[20] Harvey HA, Ketterer MR, Preston A, Lubaroff D, Williams R, Apicella MA. Ultrastructural analysis of primary human urethral epithelial cell cultures infected with *Neisseria gonorrhoeae*. Infect Immun 1997;65:2420–7.

[21] Lu P, Wang S, Lu Y, Neculai D, Sun Q, van der Veen S. A subpopulation of intracellular *Neisseria gonorrhoeae* escapes autophagy-mediated killing inside epithelial cells. J Inf Dis 2018;219:133–44.

[22] Rice PA, Shafer WM, Ram S, Jerse AE. *Neisseria gonorrhoeae*: drug resistance, mouse models, and vaccine development. Annu Rev Microbiol 2017;71:665–86.

[23] Palmer A, Criss AK. Gonococcal defenses against antimicrobial activities of neutrophils. Trends Microbiol 2018;26:1022–34.

[24] Veale DR, Finch H, Smith H, Witt K. Penetration of penicillin into human phagocytes containing *Neisseria gonorrhoeae*: intracellular survival and growth at optimum concentrations of antibiotic. Microbiol 1976;95:353–63.

[25] Johnson MB, Criss AK. *Neisseria gonorrhoeae* phagosomes delay fusion with primary granules to enhance bacterial survival inside human neutrophils. Cell Microbiol 2013;15:1323–40.

[26] Johnson MB, Criss AK. Resistance of *Neisseria gonorrhoeae* to neutrophils. Front Microbiol 2011;2:77.

[27] Quillin SJ, Seifert HS. *Neisseria gonorrhoeae* host adaptation and pathogenesis. Nat Rev Microbiol 2018;16:226–40.

[28] Birrell JM, Gunathilake M, Singleton S, Williams S, Krause V. Characteristics and impact of disseminated gonococcal infection in the “Top End” of Australia. Am J Trop Med Hyg 2019;101:753–60.

[29] Liu P, Derendorf H. Antimicrobial tissue concentrations. Infect Dis Clin North Am 2003;17:599–613.

[30] Seral C, Carryn S, Tulkens PM, Van Bambeke F. Influence of P-glycoprotein and MRP efflux pump inhibitors on the intracellular activity of azithromycin and ciprofloxacin in macrophages infected by *Listeria monocytogenes* or *Staphylococcus aureus*. J Antimicrob Chemother 2003;51:1167–73.

[31] Matzneller P, Krasniqi S, Kinzig M, Sörgel F, Hüttner S, Lackner E, et al. Blood, tissue, and intracellular concentrations of azithromycin during and after end of therapy. Antimicrob Agents Chemother 2013;57:1736–42.

[32] Barcia-Macay M, Seral C, Mingeot-Leclercq M-P, Tulkens PM, Van Bambeke F. 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] Bongers S, Hellebrekers P, Leenen LPH, Koenderman L, Hietbrink F. Intracellular penetration and effects of antibiotics on *Staphylococcus aureus* inside human neutrophils: a comprehensive review. Antibiotics (Basel) 2019;8:E54.

[34] Bish SE, Song W, Stein DC. Quantification of bacterial internalization by host cells using a beta-lactamase reporter strain: *Neisseria gonorrhoeae* invasion into cervical epithelial cells requires bacterial viability. Microbe. Infect 2008;10:1182–91.

[35] Pea F. Intracellular pharmacokinetics of antibacterials and their clinical implications. Clin Pharmacokinet 2018;57:177–89.

[36] Van Bambeke F, Barcia-Macay M, Lemaire S, Tulkens PM. Cellular pharmacodynamics and pharmacokinetics of antibiotics: current views and perspectives. Curr Opin Drug Discov Devel 2006;9:218–30.

[37] Bosnar M, Kelnerić Z, Munić V, Eraković V, Parnham MJ. Cellular uptake and efflux of azithromycin, erythromycin, clarithromycin, telithromycin, and cethromycin. Antimicrob Agents Chemother 2005;49:2372–7.

[38] Togami K, Chono S, Morimoto K. Subcellular distribution of azithromycin and clarithromycin in rat alveolar macrophages (NR8383) in vitro. Biol Pharm Bull 2013;36:1494–9.

[39] Buyck JM, Lemaire S, Seral C, Anantharajah A, Peyrusson F, Tulkens PM, et al. In vitro models for the study of the intracellular activity of antibiotics. Methods Mol Biol 2016;1333:147–57.

[40] Michot JM, Heremans MF, Caceres NE, Mingeot-Leclercq MP, Tulkens PM, Van Bambeke F. Cellular accumulation and activity of quinolones in ciprofloxacin-resistant J774 macrophages. Antimicrob Agents Chemother 2006;50:1689–95.

[41] Carryn S, Chanteux H, Seral C, Mingeot-Leclercq MP, Van Bambeke F, Tulkens PM. Intracellular pharmacodynamics of antibiotics. Infect Dis Clin North Am 2003;17:615–34.

[42] Kong FYS, Horner P, Unemo M, Hocking JS. Pharmacokinetic considerations regarding the treatment of bacterial sexually transmitted infections with azithromycin: a review. J Antimicrob Chemother 2019;74:1157–66.

[43] Taylor SN, Morris DH, Avery AK, Workowski KA, Batteiger BE, Tiffany CA, et al. Gepotidacin for the treatment of uncomplicated urogenital gonorrhea: a phase 2, randomized, dose-ranging, single-oral dose evaluation. Clin Infect Dis 2018;67:504–12.

[44] Taylor SN, Marrazzo J, Batteiger BE, Hook EW, Seña AC, Long J, et al. Single-dose zoliflodacin (ETX0914) for treatment of urogenital gonorrhea. N Engl J Med 2018;379:1835–45.

[45] Ross JDC, Brittain C, Cole M, Dewsnap C, Harding J, Hepburn T, et al. Gentamicin compared with ceftriaxone for the treatment of gonorrhoea (G-ToG): a randomised non-inferiority trial. Lancet 2019;393:2511–20.

[46] Barbee LA. Preparing for an era of untreatable gonorrhea. Curr Opin Infect Dis 2014;27:282–7.

[47] Igawa G, Yamagishi Y, Lee K-I, Dorin M, Shimuta K, Suematsu H, et al. *Neisseria cinerea* with high ceftriaxone mic is a source of ceftriaxone and cefixime resistance-mediating penA sequences in *Neisseria gonorrhoeae*. Antimicrob Agents Chemother 2018;62. e02069–17.

[48] Hook III EW, Bernstein K. Kissing, saliva exchange, and transmission of *Neisseria gonorrhoeae*. Lancet Inf Dis 2019;19. e367–e9.

[49] Barbee LA, Soge OO, Morgan J, Leclair A, Bass T, Werth BJ, et al. Gentamicin alone is inadequate to eradicate *Neisseria gonorrhoeae* from the pharynx. Clin Inf Dis 2019. Nov 12.

[50] Kiang TK, Ensom MH. A qualitative review on the pharmacokinetics of antibiotics in saliva: implications on clinical pharmacokinetic monitoring in humans. Clin Pharmacokinet 2016;55:313–58.

[51] Scangarella-Oman NE, Hossain M, Dixon PB, Ingraham K, Min S, Tiffany CA, et al. Microbiological analysis from a phase 2 randomized study in adults evaluating single oral doses of gepotidacin in the treatment of uncomplicated urogenital gonorrhea caused by *Neisseria gonorrhoeae*. Antimicrob Agents Chemother 2018;62. e01221–18.

[52] Bulitta JB, Hope WW, Eakin AE, Guina T, Tam VH, Louie A, et al. Generating robust and informative nonclinical *in vitro* and *in vivo* bacterial infection model efficacy data to support translation to humans. Antimicrob Agents Chemother 2019;63:e02307–18.

[53] Drusano GL. Pre-clinical in vitro infection models. Curr Opin Pharmacol 2017;36:100–6.

[54] Foerster S, Drusano G, Golparian D, Neely M, Piddock LJV, Alirol E, et al. In vitro antimicrobial combination testing of and evolution of resistance to the first-in-class spiropyrimidinetrione zoliflodacin combined with six therapeutically relevant antimicrobials for *Neisseria gonorrhoeae*. J Antimicrob Chemother 2019;74:3521–9.

[55] Basarab G, Kern G, McNulty J, Mueller JP, Lawrence K, Vishwanathan K, et al. Responding to the challenge of untreatable gonorrhea: ETX0914, a first-in-class agent with a distinct mechanism-of-action against bacterial Type II topoisomerases. Sci Rep 2015;5:11827.

[56] VanScoy BD, Fikes F, Bhavnani SM, Conde H, Scangarella-Oman NE, Ambrose PG, editors. A hollow-fiber infection model to evaluate the prevention of on-therapy resistance of *Neisseria gonorrhoeae* to gepotidacin. STD Prevention Conference; 27–30 August 2018; Washington DC; 2018.

[57] Edwards JL, Butler EK. The pathobiology of *Neisseria gonorrhoeae* lower female genital tract infection. Front Microbiol 2011;2:102.

[58] Yu Q, Wang L-C, Di Benigno S, Gray-Owen SD, Stein DC, Song W. *Neisseria gonorrhoeae* infects the heterogeneous epithelia of the human cervix using distinct mechanisms. PLOS Pathog 2019;15:e1008136.

[59] Carryn S, Van Bambeke F, Mingeot-Leclercq M-P, Tulkens PM. Comparative intracellular (THP-1 macrophage) and extracellular activities of β-lactams, azithromycin, gentamicin, and fluoroquinolones against *Listeria monocytogenes* at clinically relevant concentrations. Antimicrob Agents Chemother 2002;46:2095–103.

[60] Mallegol J, Fernandes P, Seah C, Guyard C, Melano RG. Determination of *in vitro* activities of solithromycin at different pHs and its intracellular activity against clinical isolates of *Neisseria gonorrhoeae* from a laboratory collection. Antimicrob Agents Chemother 2013;57:4322–8.

[61] Łaniewski P, Gomez A, Hire G, So M, Herbst-Kralovetz MM. Human three-dimensional endometrial epithelial cell model to study host interactions with vaginal bacteria and *Neisseria gonorrhoeae*. Infect Immun 2017;85. e01049–16.

[62] Raterman EL, Jerse AE. Female mouse model of *Neisseria gonorrhoeae* infection. In: Christodoulides M, editor. *Neisseria gonorrhoeae*: methods and protocols. New York, NY: Springer New York; 2019. p. 413–29.

[63] Connolly KL, Eakin AE, Gomez C, Osborn BL, Unemo M, Jerse AE. Pharmacokinetic data are predictive of *in vivo* efficacy for cefixime and ceftriaxone against susceptible and resistant neisseria gonorrhoeae strains in the gonorrhea mouse model. Antimicrob Agents Chemother 2019;63. e01644–18.

[64] Chisholm SA, Mouton JW, Lewis DA, Nichols T, Ison CA, Livermore DM. Cephalosporin MIC creep among gonococci: time for a pharmacodynamic rethink? J Agents Chemother 2010;65:2141–8.

[65] Soda M, Ito S, Matsumaru N, Nakamura S, Nagase I, Takahashi H, et al. Evaluation of the microbiological efficacy of a single 2-gram dose of extended-release azithromycin by population pharmacokinetics and simulation in Japanese patients with gonococcal urethritis. Antimicrob Agents Chemother 2017;62:e01409–17.

[66] Hook III EW, Newman L, Drusano G, Evans S, Handsfield HH, Jerse AE, et al. Development of new antimicrobials for urogenital gonorrhea therapy: clinical trial design considerations. Clin Inf Dis 2020;70:1495–500.