Narrative review

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

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A B S T R A C T

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 therapeutics.

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

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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 cefepime 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.

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 extra-cellular (intestinal 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 *in vivo* models remains 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 physico-chemical 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 ≥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 urethral 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 ceftriaxone and cefoxitin against susceptible and resistant *N. gonorrhoeae* strains based on the PK/PD index for β-lactams (\( \text{AUC} > \text{MIC} \)) 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 (\( \text{AUC} > \text{MIC} \) 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 \( \text{AUC} > \text{MIC} \) 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 \( \text{AUC} > \text{MIC} \) for at least 20–24 hours [63]. In contrast, there is a dose-dependent response for ciprofloxacin with an fAUC/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>
<thead>
<tr>
<th><strong>Site of infection</strong></th>
<th>Study relevance of intracellular location and antibiotic concentrations, local factors, e.g. biofilm, protein binding, bacterial burden, clumping, influence of commensals, immune system</th>
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<tr>
<td>Relevant antibiotic concentration</td>
<td>Which concentrations to use for modelling: do serum concentrations reflect the concentrations required at urogenital, rectal and pharyngeal sites of infection?</td>
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<tr>
<td>PK/PD index</td>
<td>Adapt PK/PD indices that consider single-dose and multiple-dose regimens and the requirement for sterilization</td>
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<tr>
<td>Strain-dependent factors</td>
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<td>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</td>
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<td>Develop in vivo models for infections other than cervical gonorrhoea, expand studies and correlate the results with known clinical outcome of old antibiotics</td>
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<td>Clinical breakpoints</td>
<td>Provide information to reassess clinical breakpoints, define failure thresholds</td>
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<td>Dosing regimens</td>
<td>Explore different dosing regimens: single-dose, multiple-dose and combination therapy</td>
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<td>Resistance</td>
<td>Integrate the goals of fast killing with minimized emergence of resistance</td>
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<td>Research environment</td>
<td>Intensify international collaborative actions and research efforts</td>
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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 \( N. \text{gonorrhoeae} \) 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.

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**References**


**Table 2**

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<tr>
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**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.


