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Treatment of human challenge and MDR strains of *Neisseria gonorrhoeae* with LpxC inhibitors

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Objectives: Inhibitors of UDP-3-*O*-(*R*-3-hydroxymyristoyl)-*N*-acetylglucosamine deacetylase (LpxC), which catalyses the second step in the biosynthesis of lipid A, have been developed as potential antibiotics for Gram-negative infections. Our objectives were to determine the effect of LpxC inhibition on the *in vitro* survival and inflammatory potential of *Neisseria gonorrhoeae*.

Methods: Survival of four human challenge strains was determined after treatment with two LpxC inhibitors for 2 and 4 h. To confirm results from treatment and assess their anti-inflammatory effect, the expression of TNF-α by human THP-1 monocytic cells infected with bacteria in the presence of the LpxC inhibitors was quantified. Cytotoxicity of inhibitors for THP-1 cells was evaluated by release of lactate dehydrogenase. Survival of five MDR strains was determined after 2 h of treatment with an LpxC inhibitor and the effect of co-treatment on MICs of ceftriaxone and azithromycin was examined.

Results: The inhibitors had bactericidal activity against the four human challenge and five MDR strains with one compound exhibiting complete killing at ≥5 mg/L after either 2 or 4 h of treatment. Treatment of gonococci infecting THP-1 monocytic cells reduced the levels of TNF-α probably owing to reduced numbers of bacteria and a lower level of expression of lipooligosaccharide. Neither inhibitor exhibited cytotoxicity for THP-1 cells. The MIC of azithromycin was slightly lowered by sublethal treatment of two MDR strains with an LpxC inhibitor.

Conclusions: Our *in vitro* results demonstrated promising efficacy of LpxC inhibition of *N. gonorrhoeae* that warrants further investigation particularly owing to the rise in MDR gonorrhoea.

Introduction

Gonorrhoea represents a growing burden of disease worldwide. The WHO estimates there are >106 million cases of gonorrhoea annually[1] and the reported incidence of gonococcal infection in countries with good surveillance has been increasing. For example, cases of gonorrhoea increased by 90% in Australia from 2009 to 2014[2] and by 11% in the USA from 2014 to 2015.[3] Gonorrhoea affects countries of all income levels with the highest rates reported in some African countries where there are 50 and 100 new infections per 1000 women and men, respectively, each year.[1] In addition, a growing number of studies have shown that gonococcal infection can facilitate susceptibility to and transmission of HIV.[4–6]

In men, infection manifests most commonly as urethritis, but as many as 40% of urogenital infections may be asymptomatic. Untreated urethral infection can lead to epididymitis, urethral stricture and reduced fertility in men. Gonorrhoea is asymptomatic in more than half of women and when present, symptoms can seem non-specific. The lack of apparent symptoms in women can lead to untreated infections and serious complications including pelvic inflammatory disease, infertility, ectopic pregnancies, spontaneous abortions, preterm birth and neonatal conjunctivitis causing scarring and blindness.

Neisseria gonorrhoeae is increasingly MDR with a real prospect that untreatable gonorrhoea could soon emerge. Lack of a vaccine for gonorrhoea further escalates this problem. Surveys of resistance in the population are the basis for treatment guidelines and when resistance to an antibiotic is >5%, its use is no longer recommended.[7] Currently only ceftriaxone and azithromycin are recommended for first-line therapy and clinical isolates resistant to both antibiotics have been reported in Denmark, Canada and Japan.[8,9] Thus, there is an urgent need for new antibiotics for gonococcal infections.[10]

The enzymes and the genes encoding them that are required for biosynthesis of the lipid A moiety of Gram-negative bacteria have been well characterized in *Escherichia coli* and are thought to present targets for the development of new antibiotics. The first

step of lipid A biosynthesis is thermodynamically unfavourable and catalysed by the product of the *lpxA* gene that transfers an *R*-3-hydroxymyristoyl moiety to the third position of the glucosamine ring of UDP-3-*O*-*N*-acetylglucosamine.[11] UDP-3-*O*-(*R*-3-hydroxymyristoyl)-*N*-acetylglucosamine deacetylase (LpxC) is a zinc metalloamidase that catalyses the second and more thermodynamically favourable step of lipid A biosynthesis in which an acetyl moiety is cleaved from the 2-*N* of the lipid A precursor molecule to produce a free amino group that is immediately acylated with an additional *R*-3-hydroxymyristoyl fatty acid by LpxD.[12,13] Owing to the critical role of the enzyme in lipid A biosynthesis and its lack of homology with mammalian proteins, inhibitors of LpxC have been developed as potential antibiotics for the treatment of Gram-negative infections.

The first description of small molecule LpxC inhibitors, to the best of our knowledge, was by scientists at Merck in 1996.[14] Since then other groups have described inhibitors,[15–19] many of which contain a hydroxamate or another substituent that can bind the catalytic zinc cation of LpxC and a hydrophobic tail that is designed to mimic the hydroxymyristate moiety of the substrate.[20] LpxC inhibitors have been shown to be bactericidal for pathogenic Gram-negative organisms including *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Haemophilus influenzae*. Based on the relatively numerous reports of bactericidal activity against Gram-negative organisms, we hypothesized that blocking the biosynthesis of lipid A with an LpxC inhibitor could be efficacious for the treatment of gonococcal infections either by being bactericidal for the bacteria or by modulation of the host response to infection.

Inhibition of purified recombinant LpxC from *Neisseria meningitidis* has been described.[21] However, there have been no reports to date on the effect of LpxC inhibitors on survival or inflammatory potential of either *N. meningitidis* or *N. gonorrhoeae*. In this study, we evaluated the effect of LpxC inhibition on well-characterized isolates of *N. gonorrhoeae* that have been used in human challenge studies and on five strains that exhibit MDR.

Materials and methods

Bacterial strains

The MS11 and FA1090 strains of *N. gonorrhoeae* have been used in most experimental human studies of gonococcal infections over the last 25 years. MS11, a porin serotype PIB-9 strain, was isolated from a patient with anterior urethritis in 1970.[22,23] The MS11mk variant A strain descended from MS11 and was used in human challenge studies from which the variant C strain was isolated.[24] The α-chain of the oligosaccharide portion of the lipooligosaccharide (LOS) of MS11mkA is a lactose moiety whereas the MS11mkC expresses a lacto-*N*-neotetraose α-chain moiety that can be substituted with one or more *N*-acetyllactosamine groups.[25,26] FA1090 A23a, a porin serotype PIB-3 strain, was isolated in the 1970s from the endocervix of a patient who probably had a disseminated infection.[27] FA1090 has been widely used in human challenge studies[28–30] and in one of these studies the 1-81-S2 strain was isolated.[31]

The FA6140 gonococcal strain is a clinical isolate that is resistant to penicillin.[32] F89, classified as WHO Y,[8] was isolated in France from a patient with a urethral infection in 2010 and was found to have resistance to ceftriaxone and most other antibiotics tested.[33] H041, classified as WHO X, is a highly ceftriaxone-resistant isolate from the throat of a patient in Japan in 2009[34] that is considered an XDR strain owing to its extensive resistance to antimicrobials.[35,36] The 35/02 and 59/03 strains are isolates from the USA with reduced susceptibility to ceftriaxone and cefixime.[37]

Antimicrobial compounds

The LpxC inhibitors, PF-5081090 and PF-04753299, were obtained from Sigma–Aldrich (St Louis, MO, USA) through its partnership with Pfizer Global Research and Development (New London, CT, USA).

Bacterial survival assays

Bacteria were grown overnight on Difco GC agar (Becton, Dickinson and Co., Sparks, MD, USA) containing 1% BBL Isovitalex Enrichment (Becton, Dickinson and Co.). After harvesting, bacteria were diluted in warm PBS and OD at 590 nm was used to dilute serially the stocks to a concentration of 4×10^5 bacteria per mL. Total reaction volumes were 200 μL with 180 μL of RPMI 1640 medium with 10% fetal bovine serum (FBS) (Gibco, ThermoFisher, Pittsburgh, PA, USA), inoculated with 4000 bacteria in 10 μL of RPMI 1640 and with 10 μL of increasing concentrations of the LpxC inhibitors dissolved in RPMI 1640 or of the vehicle only, which was 1% DMSO in RPMI 1640. After incubation of the tubes at 37°C for 2 and 4 h, aliquots were plated in duplicate for each concentration tested. The plates were incubated overnight and then bacterial colonies were counted. Survival was expressed as the percentage of bacteria detected as cfu from aliquots of LpxC-treated bacteria compared with the cfu in aliquots of the vehicle-only treated bacterial suspensions.

Effect of inhibition of LpxC on TNF-α expression induced by gonococcal infection of THP-1 cells

The human THP-1 monocytic cell line was cultured in RPMI 1640 with 10% FBS (growth medium) at 37°C in a 5% CO_2 atmosphere. THP-1 cells were activated in growth medium containing 10 ng/mL phorbol myristate acetate (Sigma–Aldrich) for 18 h. After removing the medium and washing with PBS, the cells were trypsinized and plated in 96-well plates at 1×10^4 cells per well in growth medium. Aliquots of medium containing MS11mkA, MS11mkC, FA1090 A23a or FA1090 1-81-S2 gonococci were added to wells to achieve an moi of 1. Serial dilutions were used to prepare solutions containing the LpxC inhibitors in RPMI 1640 medium from stock solutions (5 mg/mL in DMSO) and then 5 μL aliquots were added to the wells to achieve final concentrations of 0.04, 1.0 or 25 mg/L. Cells in control wells were treated with vehicle only containing 1% DMSO. After incubating the plates at 37°C in 5% CO_2 for 18 h, cell culture supernatants were transferred to another 96-well plate and frozen at −80°C. For the detection of TNF-α in aliquots of the supernatants, an ELISA (Human TNF-α Ready-Set-Go; eBioscience, San Diego, CA, USA) was performed per the instructions of the vendor using a Thermomax (Molecular Devices, Sunnyvale, CA, USA) plate reader with detection at 450 nm.

Cytotoxicity of LpxC inhibitors for THP-1 cells

Release of lactate dehydrogenase (LDH) into cell culture supernatants was analysed to determine if the LpxC inhibitors were cytotoxic to the THP-1 cells. Cytotoxicity of polymyxin B (Sigma–Aldrich)[38–40] for the human THP-1 cells was assayed as a positive control. After activation by treatment with phorbol myristate acetate (10 ng/mL) for 18 h, the THP-1 cells were plated (1×10^4 per well) in 96-well plates in Opti-MEM medium with 1% FBS (Gibco). Once cells adhered to the wells, aliquots of the LpxC inhibitors in cell culture medium were added to create final concentrations of 0.008, 0.04, 0.2, 1.0, 5.0 or 25.0 mg/L. Aliquots of polymyxin B in RPMI 1640 were added to the wells to achieve final concentrations of 5.0, 25 and 125 mg/L. Control cells were treated with vehicle only. The plates were incubated for 37°C in 5% CO_2 for 18 h. The relative levels of LDH released from dying cells into cell culture supernatants were determined using a kit (Roche, Mannheim, Germany) with detection using a Thermomax plate reader at 490 nm.

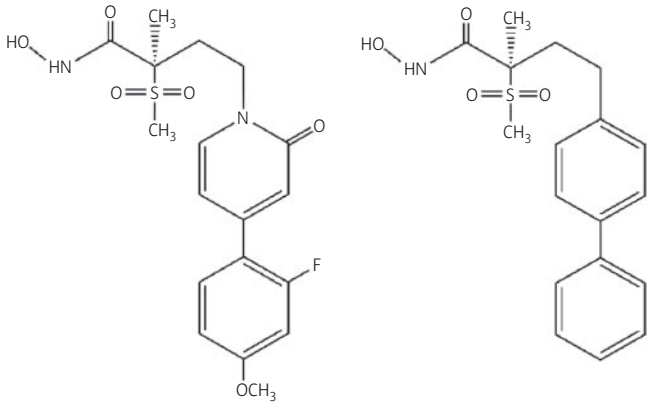

Figure 1. Structures of the two LpxC inhibitors PF-5081090 (left) and PF-04753299 (right).

Effect of LpxC inhibition on MICs for MDR strains

To determine the effect of co-treatment of the five MDR strains, absorbance at 590 nm based on comparison with a 0.5 McFarland turbidity standard (bioMérieux, Durham, NC, USA) was used to suspend the appropriate number of bacteria in 396 μL of RPMI 1640 medium with 10% FBS in microcentrifuge tubes. To achieve a final concentration of PF-04753299 of 0.2 mg/L, 4 μL aliquots of solutions of PF-04753299 in RPMI 1640 (20 mg/L) or vehicle only were added and the tubes were incubated at 37°C for 2 h. The MICs of ceftriaxone and azithromycin for the MDR strains were determined using the Etest method (bioMérieux) according to the instructions of the vendor.

Statistical analyses

SigmaPlot version 12.5 (Systat Software, San Jose, CA, USA) was used to perform statistical analyses. Multigroup comparisons were performed using one-way analysis of variance with Bonferroni's post hoc tests. Significance was defined as $P < 0.05$ for all comparisons.

Results

Activity of LpxC inhibitors against human challenge strains

The structures of the two LpxC inhibitors that were tested are shown in Figure 1. Complete killing of all four strains was observed after 4 h of treatment with PF-5081090 at a concentration of 25 mg/L and after 2 or 4 h of treatment with PF-04753299 at ≥5 mg/L (Figure 2). Only the MS11mkC isolate survived treatment with 25 mg/L PF-5081090 for the shorter 2 h period at a minimum level of 0.4% survival. The survival of the two FA1090 strains was significantly reduced by 4 h of treatment with PF-04753299 at all five concentrations tested ($P < 0.001$), whereas the MS11 strains were resistant to the lowest concentration of inhibitors tested (0.008 mg/L). The two FA1090 strains tended to be more susceptible to either inhibitor at virtually all concentrations of ≥0.2 mg/L compared with the MS11 strains. Increasing the length of treatment from 2 to 4 h reduced the survival of the FA1090 bacteria to a greater degree compared with the MS11 strains. Overall, PF-04753299 tended to be a more efficacious bactericidal compound than PF-5081090.

Effect of treatment of human challenge strains with LpxC inhibitors on inflammatory signalling of human THP-1 monocytes

LOS is a major component of the outer membrane of *N. gonorrhoeae* and is highly inflammatory. Previous work has shown that reducing phosphoethanolaminylation of the lipid A of gonococcal LOS diminishes both the inflammatory signalling and the fitness of the bacteria *in vivo*.[28,41,42] We theorized that the LpxC inhibitors could modulate inflammatory signalling induced in human THP-1 monocytic cells by killing the bacteria but also by reducing the amount of LOS expressed on the bacterial outer membrane or that was released in membrane blebs from dead or living bacteria.[43] To determine the effect of LpxC inhibition on inflammatory signalling, we incubated THP-1 cells with bacteria at an moi of 1 in the presence of the LpxC inhibitors at 0.04, 1 and 25 mg/L. As shown in Figure 3, treatment with either inhibitor at all concentrations tested resulted in a statistically significant decrease in TNF-α expression ($P < 0.01$ for all comparisons).

LpxC inhibitors are not cytotoxic for human THP-1 cells

To determine whether the LpxC inhibitors were cytotoxic at the concentrations that were bactericidal and reduced inflammatory signalling in THP-1 cells, we analysed the relative amount of LDH released from the cells following 18 h of treatment with the inhibitors. Results showed that cell death was not significantly increased by treatment with either inhibitor at the concentration range of 0.008–25 mg/L tested (Figure 4; $P > 0.05$ for all comparisons). By comparison, use of polymyxin B as a positive control resulted in a significant increase in LDH ($P < 0.001$) following treatment of THP-1 cells with 125 mg/L polymyxin B, which is a concentration similar to the MICs of polymyxin B for strains MS11 and FA1090 that have been previously reported to be 50 and 100 mg/L, respectively.[42,44] Thus, the effective concentration range of the LpxC inhibitors for gonococci does not induce cytotoxicity for THP-1 cells, whereas that for polymyxin B approaches cytotoxic concentrations.

Activity of LpxC inhibitors against MDR strains

The five MDR strains were tested with PF-04753299, which was the more efficacious of the two inhibitors. All of the MDR strains were completely killed by incubation with PF-04753299 at ≥5 mg/L and bacterial survival was <50% after incubation at 1 mg/L for 2 h (Figure 5). The susceptibility of the MDR strains was similar between strains at the concentrations tested in general except at 1.0 mg/L where, e.g. >30% of the FA6140 bacteria and <5% of the H041 bacteria survived. The MDR strains had comparable susceptibility with the human challenge strains to 2 h of treatment with PF-04753299 (Figure 2b).

Effect of LpxC inhibition on MICs for MDR strains

Co-treatment of the MDR strains with a sublethal concentration (0.2 mg/L) of PF-04753299 and ceftriaxone and azithromycin was explored using Etest strips. As shown in Table 1, in the absence of the inhibitor the MICs of the antibiotics for the MDR strains were similar to values previously reported for these strains.[32,33,35,37] In the presence of the inhibitor, no changes were observed in the susceptibility of the strains to ceftriaxone, which is a bactericidal

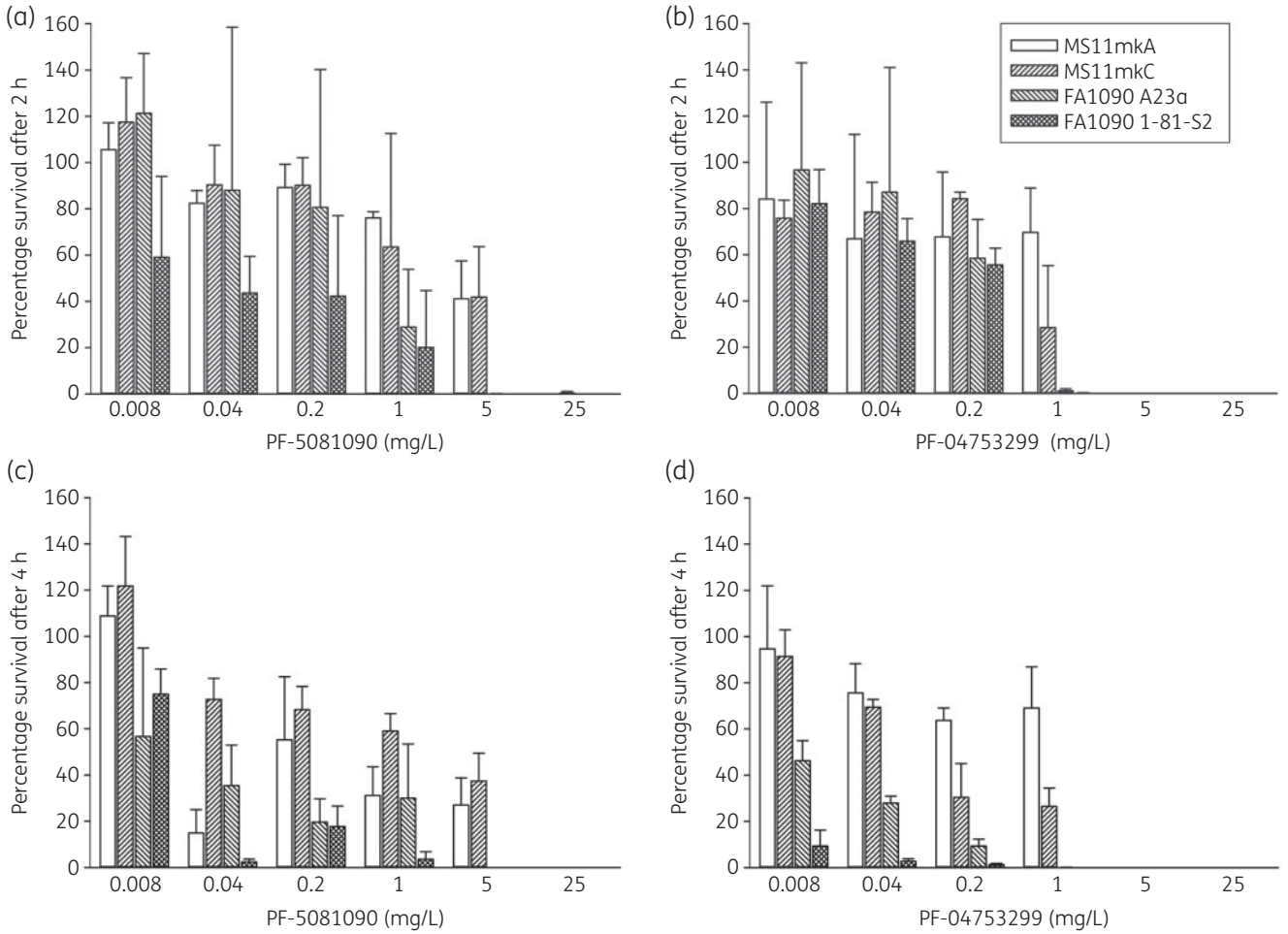

Figure 2. Bactericidal activity of the two LpxC inhibitors against the four human challenge isolates of *N. gonorrhoeae*. Increasing concentrations (0.0, 0.008, 0.04, 1.0, 5.0 or 25 mg/L) of the inhibitors were incubated with the gonococci and survival was determined after 2 and 4 h of treatment. Bars represent the mean ± SEM of triplicate experiments that were each performed in duplicate.

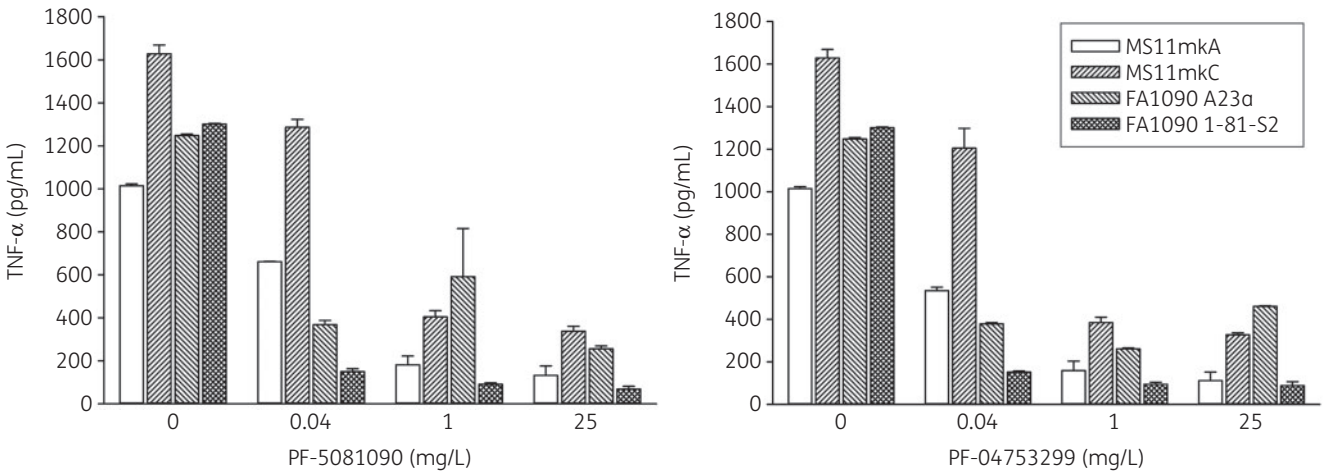

Figure 3. Levels of TNF-α in supernatants of cultures of activated human THP-1 cells infected with human gonococcal isolates at an moi of 1 in the presence of increasing concentrations (0.0, 0.04, 1.0 or 25 mg/L) of LpxC inhibitors after 18 h. Bars represent the mean ± SEM of quadruplicate experiments.

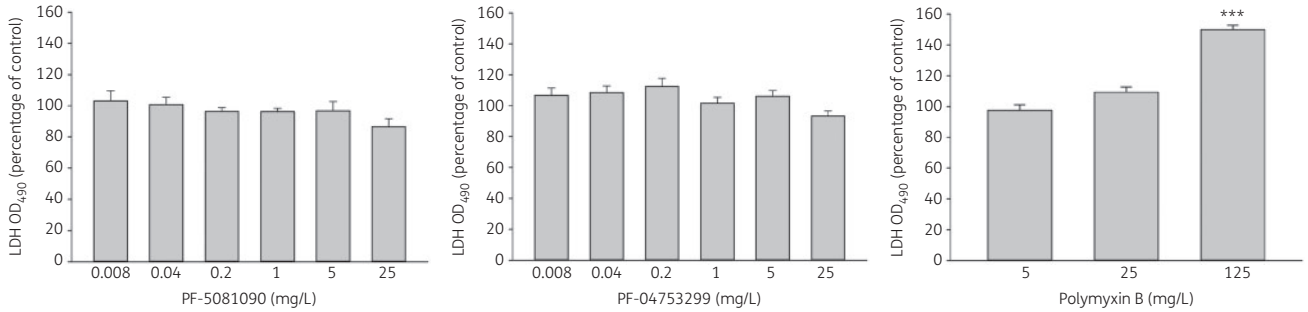

Figure 4. Relative levels of LDH in culture supernatants of activated human THP-1 cells in the presence of increasing concentrations of LpxC inhibitors (0.008–25 mg/L) or polymyxin B (5–125 mg/L) after 18 h. Bars represent the mean ± SEM of quadruplicate experiments. ***$P < 0.001$ compared with control.

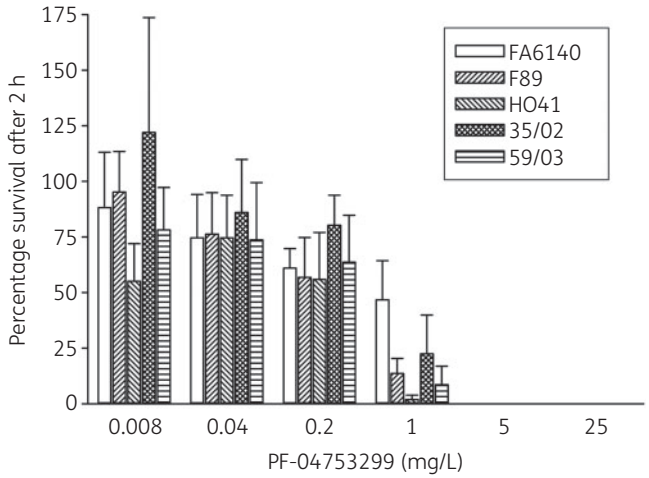

Figure 5. Bactericidal activity of PF-04753299 against five MDR strains of *N. gonorrhoeae*. Increasing concentrations of PF-04753299 (0.0, 0.008, 0.04, 1.0, 5.0 or 25 mg/L) were incubated with the gonococci and survival was determined after 2 h. Bars represent the mean ± SEM of triplicate experiments that were each performed in duplicate.

Table 1. MICs for MDR strains with and without treatment with an LpxC inhibitor

	MIC (mg/L)			
Strain	ceftriaxone	PF-04753299 + ceftriaxone	azithromycin	PF-04753299 + azithromycin
FA6140	0.032	0.032	0.19	0.125
F89	1.0	1.0	0.25	0.25
H041	1.5	1.5	0.25	0.25
35/02	0.064	0.064	0.25	0.19
59/03	0.064	0.064	0.38	0.38

MICs were determined using the Etest method, determined after treatment with PF-04753299 (0.2 mg/L) or vehicle for 2 h.

agent. However, a modest decrease in the MIC of azithromycin, which is a bacteriostatic drug, was found with co-treatment of the FA6140 and 35/02 strains. Furthermore, no antagonism from co-treatment causing a decrease in susceptibility to either drug was observed.

Discussion

Here we show that biphenyl alkyl sulphonamide hydroxamic derivative LpxC inhibitors are bactericidal for human challenge and MDR strains of *N. gonorrhoeae*. The concentrations at which PF-04753299 was bactericidal for the gonococcal isolates were comparable with the concentrations that were bactericidal for *E. coli*, *P. aeruginosa* and *K. pneumoniae* strains that had MIC_{90} values of 2, 4 and 16 mg/L, respectively, following treatment periods of 16–20 h.[15,17] However, compared with these species the gonococci were somewhat more susceptible to PF-04753299 as treatment with it at a concentration of 5 mg/L was completely bactericidal to all strains after treatment for only 2 or 4 h. We found that overall the gonococci were less susceptible to PF-5081090 compared with PF-04753299. Interestingly, a previous comparison of treatment of a panel of 106 recent clinical isolates of Gram-negative bacteria species including *P. aeruginosa* and *K. pneumoniae* with PF-5081090 and PF-04753299 found that the former was more efficacious.[45]

The structures of LpxC orthologues vary significantly between bacterial species. Differences in the *C*-termini have been shown to alter the susceptibility to proteolytic cleavage[46] and enzymatic degradation, which could affect the rate of LPS or LOS biosynthesis[47] and, thus, sensitivity to LpxC inhibitors. There is 57% identity and 79% similarity of the primary sequence of the LpxC enzymes from *P. aeruginosa* and *E. coli*, but only 32% identity and 51% similarity of the *Aquifex aeolicus* LpxC enzyme to that of *E. coli*.[48] The structure of *N. gonorrhoeae* LpxC has 49% identity and 71% similarity to that of *E. coli*.[49] Potent LpxC inhibitors have been described, but the MICs for Gram-negative species can vary more than an order of magnitude.[15,47,50,51] Furthermore, our results show that unlike a number of other Gram-negative species that were tested with both inhibitors,[45] *N. gonorrhoeae* is more susceptible to PF-04753299 than PF-5081090. The differences in susceptibility could be owing to differences between bacterial species in the structures of the LpxC enzyme and highlight the potential importance of testing in the species of interest.

We found that treatment with LpxC inhibitors at concentrations of 0.04 mg/L or greater significantly reduced expression levels of TNF-α in an *in vitro* model of infection using a human THP-1 monocytic cell line. Induction of inflammatory signalling would be

largely due to LOS molecules in the outer membrane or in outer membrane blebs released from bacteria.[43] It is likely that the reductions in cytokine levels observed were due to reduced numbers of bacteria as well as reduced levels of LOS. Our results indicate that LpxC is a promising target for the development of new antibiotics for treating gonorrhoea as LpxC inhibitors exhibit both bactericidal and anti-inflammatory activities for *N. gonorrhoeae*.

Differences between the FA1090 and MS11 isolates have been reported including the serum sensitivity of and presence of a genetic island in MS11 and serum resistance of and absence of a genetic island in FA1090.[52,53] Furthermore, whereas MS11 does express lactoferrin-binding proteins A and B and utilizes lactoferrin, FA1090 strains do not.[52,54] A review of published studies revealed that MS11mkC has an ID_{50} for urethral infection that is nearly 2 log_{10} less than FA1090[53] and similar differences in infectivity have been observed in the mouse model of female genital infection,[55] although the reason for the differences is unclear.[53] However, the expression level of the multiple transferable resistance (Mtr) efflux pump was found to be higher in MS11 compared with FA1090.[56] MS11 has a naturally occurring mutation in the *mtr* locus that leads to the expression of a consensus element that increases the promotion of *mtrCDE* transcription and, thus, resistance to some antimicrobial compounds[57] and possibly host cationic antimicrobial peptides such as LL-37.[57] Increased expression of the Mtr efflux pump probably explains the reduced susceptibility of MS11 compared with FA1090.[56]

Our results showing that the susceptibility of the MDR gonococcal strains to PF-04753299 was very similar to that of the human challenge strains are encouraging for potential clinical applications. The data imply that mechanisms conferring resistance to LpxC inhibition and to antibiotics such as cephalosporins, fluoroquinolones and macrolides differ and, furthermore, indicate that MDR gonorrhoea may be treatable with an LpxC inhibitor.

We tested combination treatment with ceftriaxone and azithromycin as these two agents are recommended as dual therapy for gonorrhoea in current treatment guidelines.[58] More combinations of antimicrobials with LpxC inhibitors for *N. gonorrhoeae* should be tested as co-treatment with PF-04753299 showed that there was a modest decrease in the MICs of azithromycin for two of the MDR strains.

The lipid A component of LOS is highly inflammatory and the anti-inflammatory effects of LpxC inhibitors could be beneficial in treating gonorrhoea. This postulate is supported by the finding that an LpxC inhibitor that was not bactericidal for *Acinetobacter baumannii*, which can survive in the absence of LOS expression, nonetheless, protected mice from infection by reducing inflammation and facilitating phagocytosis.[59] Furthermore, treatment with the PF-5081090 LpxC inhibitor increased the susceptibility of *A. baumannii* to other antibiotics by increasing the permeability of the bacteria.[60] *N. meningitidis* and *Moraxella catarrhalis* can survive in the absence of lipid A although this was produced by directed *in vitro* mutation,[61,62] whereas *A. baumannii* LOS-deficient organisms can arise after polymyxin B treatment and have been isolated, albeit rarely, in the clinic.[63,64]

Human and animal studies show that LptA-catalysed phosphoethanolaminylation of the lipid A confers a significant survival advantage to *N. gonorrhoeae*.[28] Thus, a lower level of expression of LOS on *N. gonorrhoeae* that survived LpxC inhibitor treatment should reduce bacterial fitness and virulence. Reducing TNF-α expression in gonococcal infections in women potentially could prevent or limit damage to the fallopian tube and the sloughing of the ciliated cells that leads to infertility.[65–67] The lower inflammatory potential is a hallmark of commensal *Neisseria* species relative to the pathogenic species,[42] and surviving *N. gonorrhoeae* treated with an LpxC inhibitor could have a commensal-like, non-pathogenic relationship with its human host.

There is synergism in the clinical and epidemiological occurrence of HIV-1 and *N. gonorrhoeae*, which facilitates HIV-1 expression. The heptose monosaccharides that are absent in mammalian glycomes but present in the gonococcal LOS were found to drive HIV-1 expression and innate immune responses.[68] Furthermore, recent data show that vaginal inflammation facilitates the transmission of HIV.[69] Thus, treatment of gonorrhoea with an LpxC inhibitor that not only kills the bacteria but also reduces its expression of LOS and capacity for inflammatory signalling potentially could limit the transmission of HIV by coinfected individuals.

Ideally, new antimicrobial agents for gonorrhoea would have activity against other coinfecting sexually transmitted pathogens such as *Chlamydia trachomatis*.[70] Small molecule inhibitors of LpxC were not bactericidal for *C. trachomatis*, but did block LPS synthesis and the transition of reticulate bodies to elementary bodies, a form that is required for the invasion of mammalian cells.[71]

Overall, our results showing the bactericidal effects on human challenge isolates and MDR strains of *N. gonorrhoeae* indicate that further studies of LpxC inhibitors as treatments for gonococcal infections are warranted, particularly as there is an urgent need for new antibiotics for gonorrhoea because of the rise in MDR strains. Furthermore, owing to the likely ability of LpxC inhibitors to reduce the expression of LOS on viable gonococci and, thus, impair bacterial fitness, testing in an *in vivo* model of gonococcal infection potentially could reveal efficacy that would be even greater than that observed in *in vitro* experimentation.

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Transparency declarations

None to declare.

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