David R. Cameron, Vincent Gerusz, Guennaëlle Dieppois, Zrinka Ivezic-Schoenfeld

GOFABICIN

A new FabI-inhibitor targeting antibiotic-resistant *Neisseria gonorrhoeae*

Background

Gonorrhea is the second most common sexually transmitted infection with 82 million cases per year globally.[1] The problem of *Neisseria gonorrhoeae* infection is compounded by the emergence of strains resistant to current first-line treatments, ceftriaxone and azithromycin. Development of new antibiotics that are not impacted by cross-resistance to existing treatments is crucial, and likely best achieved by exploiting new targets and modes of action.

>>> An untapped antimicrobial target for the treatment of gonorrhea is the enoyl-ACP reductase enzyme, FabI, that is essential for fatty acid biosynthesis in *N. gonorrhoeae*[2]

Gofabicin is a FabI-Inhibitor Tailored for Activity Against *N. gonorrhoeae*

Starting from the lead compound, Debio 1452, medicinal chemistry was guided by structure activity relationships (SAR) and structure-based drug design, delivering novel FabI-inhibitors with improved activity against *N. gonorrhoeae* FabI (*Ng*FabI), including Compound 1 (inhibitory concentration 50% (IC_{50}) 6 nM) and gofabicin.

>>> Gofabicin displayed potent inhibitory activity against *N. gonorrhoeae* FabI (IC_{50} = 0.6 nM)

Minimum inhibitory concentrations (MIC) were generated against 14 *N. gonorrhoeae* isolates using agar dilution according to Clinical Laboratory Standards Institute (CLSI) guidelines (M07). Decreases in IC_{50} for *Ng*FabI was generally parallelled by decreased MIC **(Figure 1)**.

>>> Gofabicin showed 16 to 64-fold increased anti-gonococcal activity as compared to the lead compound, Debio 1452 (Figure 1)

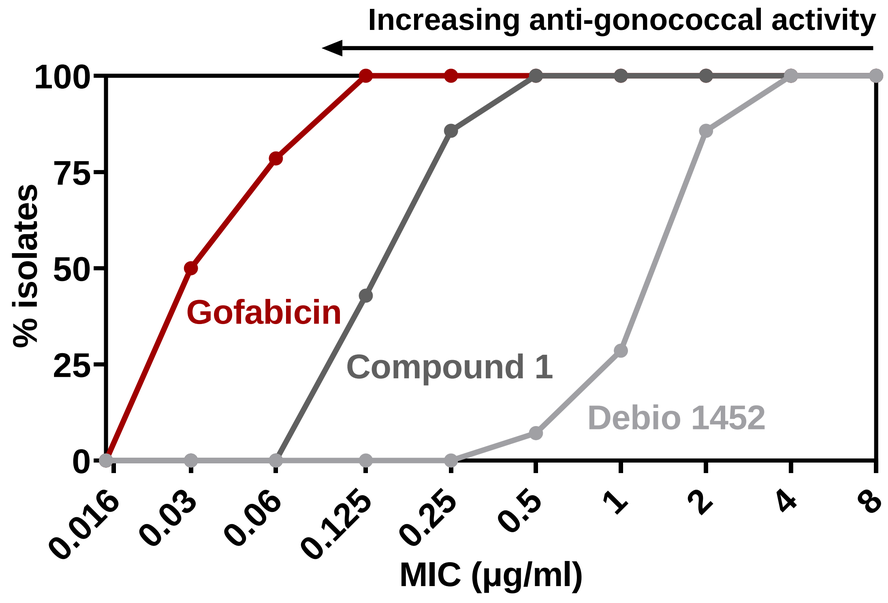

Figure 1. Cumulative MIC distribution

Gofabicin is Rapidly Bactericidal Against Antibiotic-Resistant *N. gonorrhoeae*

To assess the in vitro killing kinetics of gofabicin against *N. gonorrhoeae* in liquid culture, MICs for a panel of 10 isolates were first determined using broth microdilution according to CLSI guidelines (M07) with substitution of cation-adjusted Mueller Hinton broth (CA-MHB) media for Columbia broth. MICs in liquid were similar to those generated using agar-dilution (within one 2-fold dilution, data not shown).

>>> MICs for gofabicin ranged from 0.03 to 0.125 µg/ml against *N. gonorrhoeae* including those resistant to ceftriaxone (CTX), azithromycin (AZI) and/or ciprofloxacin (CIP) (Table 1; resistant values in red)

Bactericidal activity was assessed using the Time-Kill method according to CLSI guidelines (M26-A) with substitution of CA-MHB media for Columbia broth and determination of colony forming units (CFU) on GC agar. Ceftriaxone and azithromycin were included as controls for ATCC 49226 and displayed characteristic time-kill profiles[3] (data not shown). Gofabicin was tested against each of the ten strains listed in **Table 1**.

>>> Gofabicin was rapidly bactericidal, producing ≥3 log_{10}CFU reductions within 24h for each of the ten isolates (Figure 2)

The mean time to reach bactericidal activity across the 10 isolates (shown in Table 1) was 10 hours at 2X MIC, 8.5 hours at 4X MIC, 8 hours at 8X MIC and 8 hours at 16X MIC suggesting time-dependent, rather than concentration-dependent, killing in vitro.

Table 1. MICs (µg/ml) in liquid media.

Isolate	CTX	AZI	CIP	Gofabicin
ATCC 49226	0.004	0.25	0.004	**0.125**
ATCC 700825	0.002	0.032	0.004	**0.03**
6926	0.004	0.12	0.008	**0.06**
6804	0.016	0.25	**>1**	**0.06**
AR Bank-0157	0.004	**4**	0.004	**0.06**
AR Bank-0179	0.004	**4**	0.004	**0.06**
WHO V	0.016	**>16**	**>1**	**0.125**
WHO X	**1**	0.25	**>1**	**0.125**
WHO Y	**0.5**	0.5	**>1**	**0.125**
WHO Z	**0.25**	0.5	**>1**	**0.125**

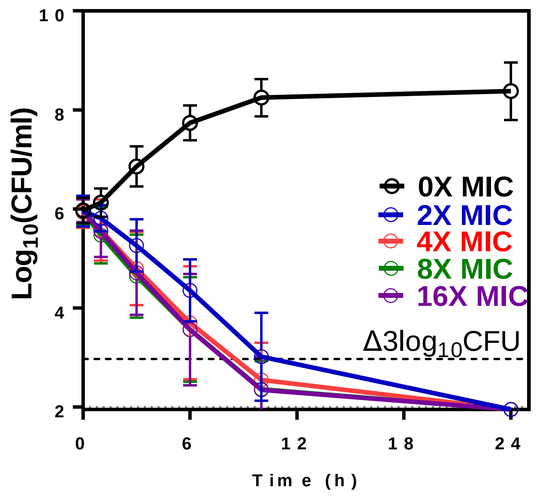

Figure 2. Time-Kill curves for gofabicin and 10 *N. gonorrhoeae* strains.
Data are the mean of the 10 isolates +/- SD. The y-axis begins at the limit of detection.

Gofabicin Kills Intracellular *N. gonorrhoeae*

N. gonorrhoeae invades and persists within cells of the human genital mucosa. New antibiotics for the treatment of gonorrhea should be capable of killing internalized *N. gonorrhoeae* cells. The capacity for gofabicin to kill intracellular *N. gonorrhoeae* was assessed within cultured HeLa229 human cervix carcinoma cells as described previously[4] for two strains, ATCC 49226 and the antibiotic-resistant strain WHO X.

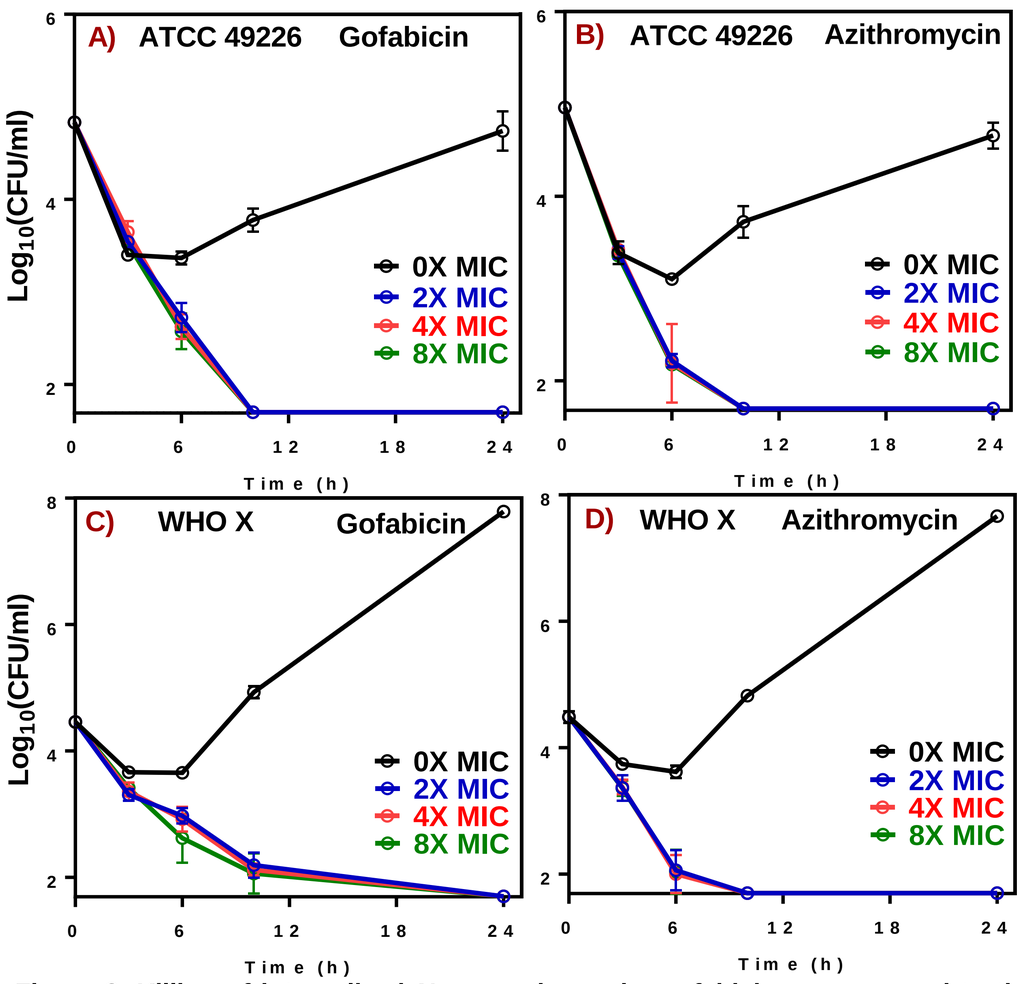

Figure 3. Killing of internalized *N. gonorrhoeae* by gofabicin as compared to the control antibiotic, azithromycin.
The y-axis begins at the limit of detection. Data are mean of three replicates +/- SD.

>>> Gofabicin eradicated intracellular *N. gonorrhoeae* to the limit of detection for both strains within 24 hours (Figure 3A, C)

The reduction from baseline at 24 hours for ATCC 49226 and WHO X strains was ≥3.1 log_{10}CFU/ml and ≥2.8 log_{10}CFU/ml, respectively.

Intracellular killing kinetics were similar to the positive control, azithromycin **(Figure 3B, D)**.

CONCLUSION

Gofabicin is a novel FabI-inhibitor for *N. gonorrhoeae* with potent in vitro bactericidal activity against antibiotic-resistant strains.

REFERENCES

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4) Mallegol et al. AAC (2013);**57**:4322-4328

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david.cameron@debiopharm.com