ICAAC ICC2015
San Diego, California | September 17-21

Poster A029

Activity of the Novel Bacterial Type II Topoisomerase Inhibitor GSK2140944 (Gepotidacin) Against Extracellular and Intracellular Forms of Susceptible and Resistant *S. aureus:* Comparison with Moxifloxacin, Ciprofloxacin, Linezolid, Clarithromycin, and Daptomycin

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Abstract (updated)

Background:

GSK2140944 selectively inhibits bacterial DNA replication through a unique binding mode towards type II topoisomerase and shows *in vitro* activity against *S. aureus* isolates resistant to fluoroquinolones. It is presently in a phase II clinical trial for treatment of acute bacterial skin and skin structure infections. Our aim was to assess its intracellular activity against intracellular forms of *S. aureus* with different resistance phenotypes to currently used antistaphylococcal agents.

Methods:

MICs were determined according to CLSI recommendations and resistance interpreted using EUCAST and CLSI/FDA interpretive criteria. Extracellular (broth) and intracellular (THP-1 monocytes) activities were measured after 24 h exposure to a wide range of concentrations (0.03 to 100 x MIC for susceptible strains) as described in AAC 2006, 5:841-851, allowing for determination of key pharmacodynamic parameters (E_{max} [maximal relative efficacy] and C_s [static concentration] as means of 3 independent experiments each ran in triplicate)

Results:

GSK2140944 had similar MICs (0.25-1 mg/L) against all strains disregarding resistance mechanisms to other drugs and was bactericidal in broth. The table shows the MICs and the pertinent pharmacodynamic parameters for intracellular activity. GSK2140944 had E_{max} values similar to other drugs (except MXF) and was more potent (lowest C_s value [in mg/L]) against resistant strains, including that expressing the ciprofloxacin transporter *norA*.

Strain	Antibiotic [a]	MIC (mg/L) [f]	Intracellular activities (with 95% confidence interval)		
			E_{max} [g]	C_s [h]	
				mg/L	X MIC
ATCC25923 [a]	GSK2140944	0.5-1	-1.07 (-1.36 to -0.78)	0.40 (0.22 to 0.58)	0.80 (0.44 to 1.17)
	LZD	2	-0.78 (-0.99 to -0.57)	4.60 (4.40 to 4.79)	2.30 (2.20 to 2.40)
	CLR	0.25	-0.38 (-0.73 to -0.04)	1.23 (1.10 to 1.34)	4.90 (4.42 to 5.37)
	DAP	1	-0.79 (-1.19 to -0.40)	2.35 (1.03 to 3.67)	2.35 (1.03 to 3.67)
	MXF	0.03-0.0625	-1.70 (-2.11 to -1.30)	0.03 (0.03 to 0.04)	1.10 (0.95 to 1.25)
MU50 [b]	GSK2140944	0.25	-1.60 (-1.92 to -1.26)	0.16 (0.13 to 0.20)	0.65 (0.52 to 0.79)
	CLR	>256 *	1.98 (1.89 to 2.07)	No convergence [i]	No convergence [i]
	DAP	8 *	-0.70 (-0.93 to -0.47)	16.3 (15.20 to 18)	2.04 (1.90 to 2.23)
	MXF	4 *	-2.42 (-2.91 to -1.93)	3.81 (3.75 to 3.88)	0.95 (0.94 to 0.97)
SA1 [c]	GSK2140944	0.5	-0.79 (-1.04 to -0.54)	0.59 (0.31 to 0.88)	1.19 (0.62 to 1.75)
	MXF	0.0625	-1.29 (-1.62 to -0.96)	0.10 (0.08 to 0.11)	1.52 (1.30 to 1.74)
	CIP	4 *	-1.25 (-1.64 to -0.85)	3.36 (2.97 to 3.76)	0.84 (0.74 to 0.94)
NRS119 [d]	GSK2140944	0.5-1	-1.14 (-1.42 to -0.85)	0.42 (0.34 to 0.51)	0.84 (0.67 to 1.01)
	LZD	64 *	-1.31 (-1.82 to -0.81)	69.3 (47.1 to 96)	1.08 (0.74 to 1.50)
	DAP	2 *	-0.44 (-0.67 to -0.20)	3.85 (3.61 to 4.10)	1.92 (1.80 to 2.05)
	MXF	4 *	-2.11 (-2.54 to -1.68)	4.50 (3.12 to 5.88)	1.12 (0.78 to 1.47)

[a] Laboratory Standard (ATCC; Manassas, VA)
[b] ATCC700699 (Manassas, VA)
[c] Ba *et al* (2006) Antimicrob Agents Chemother 50:1931–6
[d] Tsiodras et al (2001) The Lancet 358: 207-208
[e] LZD: linezolid; CLR: clarithromycin; DAP: daptomycin; MXF: moxifloxacin ; CIP : ciprofloxacin
[f] values with * are considered as denoting non-susceptibility according to the 2015 EUCAST and the 2014 CLSI (LZD, CLR, DAP) or FDA (DAP) interpretive criteria
[g] CFU count decrease (in $\log_{10}$ units) at 24 h from the corresponding initial inoculum as extrapolated from an infinitely high antibiotic concentration; values are means (95% confidence intervals) of 3 independent determinations
[h] extracellular concentration resulting in no apparent bacterial growth (number of CFU identical to the initial inoculum), as calculated from the Hill equation of the concentration-response curve; values are means (95% confidence intervals) of 3 independent determinations
[i] no significant CFU count decrease over the range of concentrations tested (0.7-256 mg/L)

Conclusions:

GSK2140944 extracellular and intracellular activities remain unaffected by resistance mechanisms to currently used antistaphylococcal agents. Since intracellular C_ss for GSK2140944 closely match its MICs in broth, the results are consistent with a penetration of GSK2140944 into cells. The data suggest that GSK2140944 warrants further study for the treatment of staphylococcal infections, including those caused by resistant isolates.

References

1. Hawkey & Jones (2009) J Antimicrob Chemother 64 Suppl 1:i3-10. PubMed: 19675017.
2. Garzoni & Kelley (2009) Trends Microbiol 17:59-65. PubMed: 19208480
3. Performance Standards for Antimicrobial Susceptibility Testing; 24d Informational Supplement. CLSI document M100-S24. Wayne, PA: Clinical and Laboratory Standards Institute; 2013.
4. Barcia-Macqay *et al.* (2006) Antimicrob Agents Chemother 50:841-51. PubMed: 16495241
5. Hoover *et al.* (2013) 53d Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), abstract A-014.

Funding

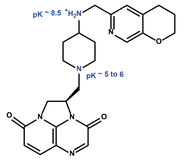
This work was supported by GlaxoSmithKline and has been funded in whole or in part with Federal funds from the Office of the Assistant Secretary for Preparedness and Response, Biomedical Advanced Research and Development Authority, under Contract No. HHSO100201300011C. F.P. is paid by the *Université catholique de Louvain*, F.V.B. is Senior Research Associate of the Belgian *Fonds de la Recherche Scientifique* (F.R.S.-FNRS), P.M.T. is emeritus and unpaid invited Professor.

Background and Aims

Staphylococcus aureus remains a therapeutic challenge, due in part to the ability of this organism to acquire resistance mechanisms to most recommended antibiotics [1] and to survive in intracellular compartments of eukaryotic cells [2]. In this context, it is therefore essential (i) to foster the discovery and development of novel antibiotics with mode(s) of action distinct from those in current use, and (ii) to assess the activity of these molecules against intracellular *S. aureus*.

Gepotidacin (formerly GSK2140944 - see hereunder) is a novel antimicrobial agent belonging to the chemical class of bacterial topoisomerase type II inhibitors (BTIs), which are structurally different from fluoroquinolones. Gepotidacin selectively inhibits bacterial DNA replication through a unique binding mode towards type II topoisomerase, therefore bypassing resistance to established antibiotics including fluoroquinolones. Gepotidacin is presently in phase II of clinical development for treatment of acute bacterial skin and skin structure infections (ABSSSIs).

The aim of our study was to assess and measure the activity of Gepotidacin against extra- and intracellular forms of *S. aureus* in comparison with other currently used anti-staphylococcal antibiotics using strains with different resistance mechanisms to these antibiotics.



Gepotidacin (formerly GSK2140944)
Preferred IUPAC name: (3R)-3-[[4-[(2H,3H,4H-pyrano[2,3-c]pyridin-6-ylmethyl)amino]piperidin-1-yl]methyl]-1,4,7- triazatricyclo[6.3.1.0*{4,12}]dodeca-6,8(12),9- triene-5,11-dione.

The figure shows the calculated pKa's (Reaxys) of the two aminofunctions (in blue) assumed to be ionizable in the 5-8 pH range (predominant microspecies: monoionic [68%] at pH 7.0) and dicationic [93%] at pH 5.5).
Calculated log P = -0.56 (Chemdraw) or 0.16 (Reaxys); calculated log D (Reaxys): pH7.0 = -1.71, pH 5.5 = -3.45).

Sources: structure redrawn from [5]; physicochemical constants: ChemBioDraw (http://www.cambridgesoft.com/) and Reaxys® Reed Elsevier (http://www.reaxys.com)

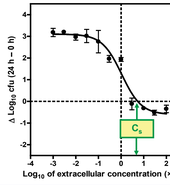
Methods

Bacterial strains and MIC determinations

- *S. aureus* reference strain ATCC25923 (MSSA) and resistant strains NRS119, MU50 and SA1 were obtained as indicated in the Table and grown in MHB as previously described [3].
- MICs were determined according to CLSI recommendations and interpreted using available CLSI clinical breakpoints [3]).

Extracellular and Intracellular activity

- Extracellular (broth) and intracellular (THP-1 monocytes, displaying macrophage-like activity) activities were measured by exposure (24h) to a wide range of concentrations (0.03 to 100-fold MIC) as previously described [4]).
- For intracellular activities, phagocytosis of opsonized bacteria was allowed for 1h using a 4:1 bacteria-macrophage ratio, followed by elimination of extracellular bacteria by 45 min exposure to gentamicin (50 mg/L).
- The increase or decrease in the number of viable bacteria was measured by colony counting of broth or cell lysates (24h incubation)
- Activities are expressed as the change in the initial inoculum at 24 h compared to the post-phagocytosis value (time 0).
- All data are means ± SEM of 3 independent experiments each ran in triplicate (when non visible, the SEM bar is smaller than the symbols)
- Data are used to fit a Hill equation allowing to determine two key pharmacological descriptors of antibiotic activity as shown here:



➢ E_{max} (maximal relative efficacy): CFU change (in $\log_{10}$ units) at 24h from the post-phagocytosis inoculum as extrapolated for an infinitely large antibiotic concentration

➢ C_s (relative potency): extracellular concentration resulting in no apparent bacterial growth from CFU at 0h (CFU at 0h = post-phagocytosis inoculum).

Results

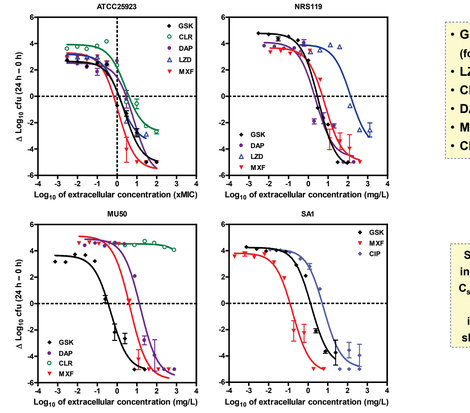
Strain	Antibiotic [b]	MIC (mg/L)	CLSI [a]
ATCC25923 [c]	Gepotidacin	0.5-1	n/a
	LZD	2	S
	CLR	0.25	S
	DAP	1	S
	MXF	0.03-0.0625	S
MU50 [d]	Gepotidacin	0.25	n/a
	CLR	>256	R
	DAP	8	R
	MXF	4	R
SA1 [e]	Gepotidacin	0.5	n/a
	MXF	0.0625	S
	CIP	4	R
NRS119 [f]	Gepotidacin	0.5-1	n/a
	LZD	64	R
	DAP	2	R
	MXF	4	R

Strains and MICs

Gepotidacin MICs ranged from 0.25-1 mg/L for all strains and were unaffected by resistance mechanisms to other antibiotics, including efflux by the ciprofloxacin efflux transporter *norA*

[a] Clinical susceptibility using CLSI interpretive criteria
n/a: not available (breakpoints not yet set)
S: susceptible (MIC ≤ than the S clinical breakpoint)
R: resistant (MIC ≥ than the R clinical breakpoint)
[b] LZD: linezolid; CLR: clarithromycin; DAP: daptomycin; MXF: moxifloxacin ; CIP : ciprofloxacin
[c] Laboratory Standard (ATCC; Manassas, VA)
[d] ATCC700699 (Manassas, VA)
[e] Ba et al (2006) Antimicrob Agents Chemother 50: 1931–6
[f] Tsiodras et al (2001) The Lancet 358: 207 - 208

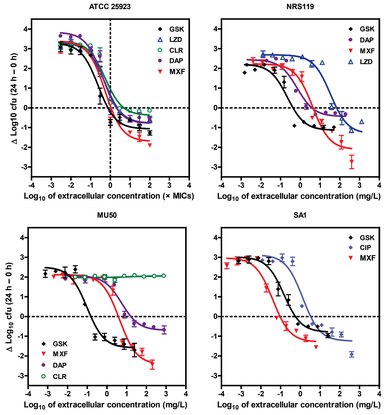
Extracellular activities



- GSK = gepotidacin (formerly GSK2140944)
- LZD = linezolid
- CLR = clarithromycin
- DAP = daptomycin
- MXF = moxifloxacin
- CIP = ciprofloxacin

- Gepotidacin is bactericidal (>99.9% inoculum decrease) for all strains with E_{max} reaching or close to the limit of detection (5 $\log_{10}$ cfu decrease) for all strains.
- The overall extracellular efficacy of gepotidacin is similar to that of moxifloxacin and daptomycin and better (more negative E_{max}) than that of linezolid and clarithromycin.
- C_s are close to the MIC in all cases, making gepotidacin more potent (on a mg/L basis) than drugs to which the strains are resistant.

Intracellular activities



See numeric data for individual intracellular C_s and E_{max} values and their confidence interval in the Table shown in the abstract

- E_{max} of all antibiotics is considerably lower (less negative) than for extracellular bacteria (note the difference in scale of the Y axis).
- The activity of gepotidacin is
  (i) unaltered by resistance to other antibiotics (similar intracellular E_{max} and C_s values for all strains),
  (ii) similar to or better than that of linezolid, clarithromycin and daptomycin, and lower (less negative E_{max}) than that of moxifloxacin.

Discussion and Conclusions

- Gepotidacin shows extracellular and intracellular activities that remain unaffected by resistance mechanisms to other antistaphyloccocal antibiotics.
- While its intracellular maximal efficacy (E_{max}) is considerably lower than its extracellular activity (as for all other antibiotics), the intracellular relative potency (C_s) of gepotidacin is close to its MICs in broth, suggesting a free penetration and an effective access to its bacterial target in phagocytes.
- Gepotidacin demonstrates extracellular and intracellular activity similar to or better (more negative E_{max}) than that of daptomycin, clarithromycin and linezolid and similar to or lower (less negative E_{max}) than that of moxifloxacin.
- These data suggest that gepotidacin may warrant further study as a potential anti-staphylococcal agent against intracellular multidrug resistant *S. aureus*.

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