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WCK 4873 (Nafithromycin): Impact of hyper *ermB* Induction in *S. pneumoniae* and *S. aureus* on the Activity of Ketolides

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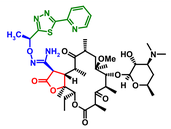
INTRODUCTION AND PURPOSE

- Macrolides/ketolides exert their antimicrobial activity by blocking the functional ribosome assembly and peptide exit channel thereby inhibiting RNA-dependent protein biosynthesis.
- Macrolide resistance in streptococci and staphylococci arises due to modification of A2058 residue in Domain V of 23S rRNA site as a result of methylation caused by inducible or constitutive *erm* (Erythromycin methyl transferase) *'A'* or *erm'B'* determinants, thus unfavorably impacting drug-target interactions[1].
- While macrolides are reported to be potent inducers of *ermB*, ketolides being non-inducers show sustained activity against macrolide resistant *erm* - expressing streptococci.
- The activity of ketolides against macrolide-resistant strains additional interaction at A752 in domain II.
- The extent of methylation plays a significant role in determining the potency of ketolides. Against mono-methylated target in SPN and SPY older ketolides cethromycin (CET) and telithromycin (TEL) show good activity. Dimethylation of the target leads to higher level resistance to CET in *S. pneumoniae* and to TEL in *S. pyogenes*.
- Nafithromycin (WCK 4873) is a novel lactone ketolide active against typical and atypical respiratory pathogens including macrolide-resistant *S. pneumoniae* (SPN), *S. pyogenes* (SPY) and methicillin susceptible staphylococci.
- There are significant strutural differences between Nafithromycin and TEL. For instance, while WCK 4873 originates from lactone ketolide nucleus, TEL is a carbamate ketolide. The lactone moiety bears a chiral carbon that helps orient the molecule in a favourable position enabling interactions with multiple 23S rRNA targets. While TEL has flexible 4 carbon spacer WCK 4873 has 4 atoms spacer containing cis double bond and chiral methyl providing a non-flexible sidechain directing the molecule's interacting moieties towards domain II of 23S rRNA. Another key difference is the terminal moity lies in the side chain, wherein TEL bears a 3-pyridine and imidazole side chain, while WCK 4873 has 2-pyridine and 1,3,4-thiadiazole side chain (Figure 1).

Figure 1: Structure of WCK 4873



WCK 4873
(NAFITHROMYCIN)

- WCK 4873 has MIC_{90} of 0.06 µg/mL against *S. pneumoniae*. WCK 4873 was also shown to be active against TEL resistant SPN isolates with MIC_{90} being 0.12 µg/mL. WCK 4873 is currently undergoing Phase 2 evaluation for CABP indication.
- In this study, we describe the activity of WCK 4873 and other ketolides against *ermB* hyper-induced SPN, SPY and staphylococci employing erythromycin (ERY) as an inducing agent.

METHOD

MIC determination

- Shift in potencies of ketolides against strains with *ermB* induction was undertaken by determining the MICs *ermB* harboring SPN, SPY and staphylococci in presence of ERY (1 µg/mL). For hyper induction, the MICs of ketolides were determined employing cultures grown in the presence of ERY (5 µg/mL) and determining the MICs in the presence of ERY. MICs of WCK 4873 and comparators TEL, CET and Solithromycin (SOL) were determined by agar dilution method using Mueller-Hinton agar supplemented with or without 5% sheep blood.

MPC studies

- For determining mutant prevention concentrations (MPCs), overnight grown *S. pneumoniae* inocula in normal saline were employed. A range of concentrations varying from 1/2× to 4× of MICs of the respective drugs were used. In order to enhance the stringency of mutant prevention assessment MPCs were also determined in presence of ERY (1 µg/mL).

RESULTS

MIC determination studies

- All isolates showed resistance to macrolides such as azithromycin and to lincosamide – clindamycin confirming the expression of *ermB*.

Activity against *S. pneumoniae*

- WCK 4873 showed lowest MICs against *ermB* harboring SPN isolates. Under standard conditions (uninduced). The $MIC_{50/90}$ for WCK 4873 was 0.03/0.12 µg/mL vs. 0.03/0.25 µg/mL for SOL. Telithromycin and CET showed four times higher MIC_{90} as compared to WCK 4873.
- In the presence of ERY, only MIC_{50} of WCK 4873 increased by just one dilution fold (0.06 µg/mL) while SOL MIC_{50} and MIC_{90} increased by eight and four times over un-induced conditions. TEL MIC_{90} was higher under both induced and uninduced conditions (0.5 µg/mL) and CET MIC_{90} increased by 8 times (0.5 to 4 µg/mL).
- Under hyper induced condition the MICs of WCK 4873 & SOL increased by 2x and 16x (MIC_{90} of WCK 4873 0.25 µg/mL vs 4 µg/mL for SOL). MIC_{90s} of TEL and CET were in the range of 1 and to 4 µg/mL, respectively.

Activity against *S. pyogenes*

- Under *ermB* induced conditions, while WCK 4873 and SOL MICs increased minimally by one dilution fold, a higher impact of induction was seen on CET and TEL MICs. (MIC_{90}s TEL 16 µg/mL and CET 1 µg/mL). These MICs increased to 32 and 4 µg/mL, respectively under hyper induced conditions. Interestingly, WCK 4873 and SOL MIC_{90}s were 1 µg/mL.

Activity against staphylococci

- For staphylococci the $MIC_{50/90}$ (µg/mL) in absence and presence of ERY were WCK 4873 – 0.12/0.25 and 4/>32, TEL – 0.25/1 and 8/>32, CET – 0.12/0.25 and 2/>32 and SOL – 0.12/0.25 and 8/>32 respectively.
- Under hyper induced condition, expectedly, for all the agents MICs increased 16 - 32 µg/mL.

Table 1: MICs of ketolides against hyper *ermB* induced *S. pneumoniae* (N =15)

Drug	MIC (µg/mL)					
	Without Erythromycin (uninduced)		Induction		Hyper induction	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
WCK 4873	0.03	0.12	0.06	0.12	0.12	0.25
TEL	0.06	0.5	0.25	0.5	0.5	1
CET	0.03	0.5	0.5	4	2	4
SOL	0.03	0.25	0.25	1	2	4

Table 2: MICs of ketolides against hyper *ermB* induced *S. pyogenes* (n=18)

Drug	MIC (µg/mL)					
	Without Erythromycin (uninduced)		Induction		Hyper induction	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
WCK 4873	0.25	0.25	0.5	0.5	0.5	1
TEL	8	8	8	16	16	32
CET	0.12	0.5	1	1	1	4
SOL	0.12	0.12	0.25	0.25	0.5	1

Table 3: MICs of ketolides against hyper *ermB* induced *staphylococci* (n=14)

Drug	MIC (µg/mL)					
	Without Erythromycin (uninduced)		Induction		Hyper induction	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
WCK 4873	0.12	0.25	4	>32	32	>32
TEL	0.25	1	8	>32	32	>32
CET	0.12	0.25	2	>32	16	>32
SOL	0.12	0.25	8	>32	16	>32

MPC determination studies

- Mutational frequency was lowest for WCK 4873 in comparison to TEL and SOL. Mutational frequency for WCK 4873 was in the range of $5X10^{-6}$ - $>2X10^{-9}$ and for SOL and TEL it was $5X10^{-4}$ - $5X10^{-6}$.
- Under standard conditions, only WCK 4873 that showed MPC at its 4X MIC. Under hyper *ermB* induction, while WCK 4873 attained MPC at 4-8X MIC, MPC was attained only at 16X MIC for SOL, and at >4-16X for TEL (with respect to their MICs under standard conditions).

Table 4: Frequency of mutation of WCK 4873 Vs comparators against *S. pneumoniae* strains

Organism (Inoculum CFU/plate)	Drug	MIC (µg/mL)	Frequency of mutation at -				MPC at fold MIC
			½	1×	2×	4×	
S. pneumoniae 3587 (2×10^9)	WCK 4873†	0.12	5×10^{-4}	5×10^{-6}	$>2\times10^{-9}$	$>2\times10^{-9}$	2X
	WCK 4873+E‡	0.12	NT	5×10^{-6}	2.08×10^{-6}	$>2\times10^{-9}$	4X
	SOL†	0.25	5×10^{-4}	5×10^{-4}	5×10^{-6}	5×10^{-6}	>4X
	SOL+E‡	1	5×10^{-4}	5×10^{-4}	5×10^{-6}	$>2\times10^{-9}$	4X
	TEL†	0.5	5×10^{-4}	5×10^{-4}	5×10^{-4}	5×10^{-4}	>4X
	TEL+E‡	0.5	5×10^{-4}	5×10^{-4}	5×10^{-4}	5×10^{-4}	>4X
S. pneumoniae 1026 (2×10^9)	WCK 4873†	0.03	5×10^{-4}	5×10^{-4}	5×10^{-6}	$>2\times10^{-9}$	4X
	WCK 4873+E‡	0.12	5×10^{-4}	5×10^{-6}	$>2\times10^{-9}$	$>2\times10^{-9}$	2X
	SOL†	0.06	5×10^{-4}	5×10^{-4}	5×10^{-4}	5×10^{-6}	>4X
	SOL+E‡	0.5	5×10^{-6}	5×10^{-6}	$>2\times10^{-9}$	$>2\times10^{-9}$	2X
	TEL†	0.06	5×10^{-4}	5×10^{-4}	5×10^{-4}	5×10^{-6}	>4X
	TEL+E‡	0.25	NT	5×10^{-4}	1.47×10^{-6}	$>2\times10^{-9}$	4X
S. pneumoniae 743 (1.15×10^9)	WCK 4873†	0.03	8.69×10^{-6}	$>1.15\times10^{-9}$	$>1.15\times10^{-9}$	$>1.15\times10^{-9}$	1X
	WCK 4873+E‡	0.12	8.69×10^{-6}	$>1.15\times10^{-9}$	$>1.15\times10^{-9}$	$>1.15\times10^{-9}$	1X
	SOL†	0.03	8.69×10^{-4}	8.69×10^{-4}	$>1.15\times10^{-9}$	$>1.15\times10^{-9}$	2X
	SOL+E‡	0.5	NT	$>1.15\times10^{-9}$	$>1.15\times10^{-9}$	$>1.15\times10^{-9}$	11X
	TEL†	0.06	8.69×10^{-6}	$>1.15\times10^{-9}$	$>1.15\times10^{-9}$	$>1.15\times10^{-9}$	1X
	TEL+E‡	0.25	8.69×10^{-6}	$>1.15\times10^{-9}$	$>1.15\times10^{-9}$	$>1.15\times10^{-9}$	1X

† MIC and MPC under standard condition
‡ MIC and MPC under Induced condition; E- Erythromycin was added at 1 µg/mL.
Viable count on drug plates with confluent growth was considered as 1×10^6 CFU/mL.

CONCLUSIONS

- **Against *ermB*-expressing SPN strains, activity of nafithromycin was the least impacted in comparison to other ketolides like SOL and TEL. indicating its better target interaction even with modified domain V and domain II of 23S rRNA.**
- **In BAL study employing WCK 4873 800 mg OD dose, the ELF concentration time profile shows that for 100% of dosing interval the WCK 4873 concentrations are above the MICs and MPCs encountered in this study.**
- **Nafithromycin has the potential for therapeutic coverage of high level macrolide and TEL resistant strains as evidenced by its potent activity against strains with hyper *ermB* induction.**

REFERENCES

1. Jerry M. Zuckerman; Infect Dis Clin N Am 18 (2004) 621–649.
2. Douthwaite et al; Molecular Microbiology (2005) 58 (2), 613–622.

DISCLOSURES:

All authors are employees of Wockhardt Research Centre, Aurangabad, India.
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