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Colistin Potentiates the Anti-bacterial Activity of 5-*O*-Mycaminosyltylonolide for Some Gram-Negative Bacteria

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We chose colistin as a new potentiator, which exhibited the relatively good potentiation activity of OMT against *Escherichia coli* and *Klebsiella pneumoniae*. This study uncovered new insight into an OMT based combination therapy.

Key words 5-*O*-mycaminosyltylonolide, Antimicrobial resistance, Gram-negative bacteria, *Escherichia coli*, *Klebsiella pneumoniae*

INTRODUCTION

Antimicrobial resistance is an ever-growing threat to humanity, being attributed to approximately 700,000 deaths annually.[1,2] The World Health Organization has published the list of antibiotic-resistant "priority pathogens," which highlights the threat of Gram-negative bacteria species that are resistant to multiple antibiotics.[3] Notably, *Klebsiella pneumoniae* (*K. pneumoniae*), *Acinetobacter baumannii* (*A. baumannii*), *Pseudomonas aeruginosa* (*P. aeruginosa*), and *Enterobacter* species are known as Gram-negative ESKAPE pathogens, which are the leading cause of nosocomial infections throughout the world.[4] Most of these pathogens have resistance to multiple antibiotics, which is one of the greatest challenges in the clinical environment. This is attributed to Gram-negative bacteria's intrinsic resistance to several antibiotics due to the impermeability of its outer membrane and the activity of several multidrug efflux pumps.[5,6] These two factors also hamper the discovery of new drug candidates. In fact, we screened active compounds from our in-house microbial secondary metabolites library for activity against Gram-negative bacteria, resulting in failure. Payne *et al*. also conducted the screening of around 500,000 compounds for activity against a Gram-negative bacterium, *Escherichia coli* (*E. coli*), but no hit compounds were identified.[7] To address the challenges, we envisioned that a combination strategy utilizing potentiators which inhibit bacterial efflux pumps or increases the permeability of the outer membrane, would lead us to discover overlooked anti-Gram-negative bacteria active compounds.[8,9] As a first step, we screened potential drug candidates against the Gram-negative bacteria, *P. aeruginosa*, from the Ōmura Natural Compound library and identified 5-*O*-mycaminosyltylonolide (OMT) (Fig. 1).[8] Moreover, OMT was found to show significant antibacterial activity against multidrug resistance (MDR) *P. aeruginosa* clinical isolates when combined with efflux pump inhibitors or polymyxin B nonapeptide (PMBN), which increases the permeability of the outer membrane.[8–10] PMBN was found to be an excellent potentiator against *P. aeruginosa*, whereas it was not effective for other Gram-negative bacteria such as *E. coli*, and *K. pneumoniae* (Table S1). In this regard, PMBN is categorized as a cationic polypeptide compound. A similar type of compound is colistin (Fig. 1), a last resort antibiotic used for MDR Gram-negative bacilli infections.[11] Therefore, our previous reports prompted us to investigate the potentiation activity of colistin for OMT against Gram-negative pathogens. In this study, we evaluated the anti-bacterial activity of OMT against Gram-negative pathogens, *E. coli*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *Enterobacter cloacae* (*E. cloacae*), in the presence of colistin as a potentiator.

MATERIALS AND METHODS

Chemicals OMT was synthesized from tylosin according to the known procedure.[12] Tylosin and colistin were purchased from FUJIFILM Wako chemicals.

Strains *E. coli* NIHJ JC-2, *K. pneumoniae* NCTC9632, *E. cloacae* IFO13535, *P. aeruginosa* PAO1, *A. baumannii*

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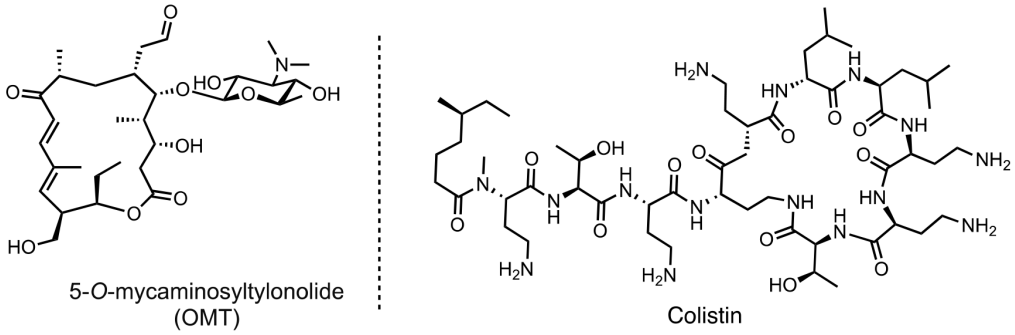

Fig. 1. Structure of OMT and Colistin

Table 1. Combination Assay with OMT and Colistin agisnt Gram-negative Bacteria

Strain		MIC (μg/mL) of each compound		MIC (μg/mL) of OMT in combination with colistin	
		OMT	Colistin	OMT + colistin[a]	OMT + colistin[b]
E. coli	NIHJ JC-2	>64	0.5	≤1	2
K. pneumoniae	NCTC9632	>64	16	4	8
E. cloacae	IFO13535	>64	32	8	8
P. aeruginosa	PAO1	>64	2	2	64
A. baumannii	ATCC19606	>64	0.5	32	64

[a] 1/2MIC represents the concentration of colistin used in the combination assay, [b] 1/4MIC represents the concentration of colistin used in the combination assay

ATCC19606, and *E. coli* ATCC25922 were used from laboratory stocked strains at Kitasato University. Five clinically isolated E. coli strains with added KUB numbers which were collected from national surveillance in Japan and were purchased via the Three Academic Societies Joint Antimicrobial Susceptibility Surveillance Program.[13] *E. coli* and *K. pneumoniae* strains of the Centers for Disease Control and Prevention and Food and Drug Administration Antibiotic Resistance Isolate Bank (AR Bank # strains) were provided by the National Institute of Infectious Diseases, Japan.

Antimicrobial Activity Minimum inhibitory concentrations (MICs) of the antimicrobial agents were determined with the broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) guideline.[14] Susceptibility testing was performed with Eiken dry plates (Eiken Chemical Co., Ltd., Tokyo, Japan).

Combination Assay The combination assay was performed using microbroth dilution method according to the Clinical Microbiology Procedures Handbook.[15]

RESULTS AND DISCUSSION

We chose *E. coli*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *E. cloacae* and conducted the combination assay to verify the potential of colistin as a potentiator for OMT (Table 1). With a quarter of the MIC value of colistin, the anti-bacterial activity of OMT against *E. coli* was significantly potentiated. *K. pneumoniae,* and *E. cloacae* showed moderate sensitivity under the same condition, whereas it was not so effective against *P. aeruginosa* and *A. baumannii*. Considering to our previous result, PMBN would be a better potentiator than colistin for *P. aeruginosa*.[9] Subsequently, we increased the concentration of colistin to half of the MIC value of colistin, which provided the lower MIC value of OMT against *K. pneumoniae.* From this investigation, *E. coli* and *K. pneumoniae* was found to display reasonable sensitivity under the combination strategy and we evaluated anti-bacterial activity against several *E. coli* and *K. pneumoniae* strains including clinical isolates (Tables 2, 3, and S2). Colistin potentiated the anti-bacterial activity of OMT against *E. coli* AR Bank#55 and *E. coli* ATCC25922 up to 8 μg/ml when using a quarter of the MIC value of colistin. It is noteworthy that the MIC value of OMT dramatically decreased (4 to 32-fold) when the use of a half the MIC value of colistin. This tendency was also seen in the case of *K. pneumoniae.* The potentiation activity of OMT by colistin was effective against some strains such *K. pneumoniae* AR Bank#523, *K. pneumoniae* AR Bank#524, and *K. pneumoniae* AR Bank#525 with even a quarter of the MIC value of colistin. In this way, we revealed that colistin displayed the relatively good potentiation activity of OMT against *E. coli* and *K. pneumoniae*, illuminating the new insight into an OMT based combination therapy. Although cationic polypeptide compounds including colistin would be one of the great options as potentiators based on these results, it is necessary that appropriate potentiators would be chosen depending on the difference of bacterial species and even strains probably due to the structural diversity of the bacterial outer membrane, which necessitates further investigations. Additionally, colistin is a well-known antibiotic that is known to have high risk for kidney injury when used in humans.[16] That is one of the reasons that colistin is a last resort antibiotic. In this regard, our approach could potentially reduce the risk of kidney injury and other serious side effects using a lower dose of colistin in combination with OMT. Toward the establishment of the OMT based combination therapy method for broad Gram-negative pathogen related infections, we are seeking several potentiators especially cationic polypeptides and planning to implement *in vivo* experiments using the mouse model of Gram-negative pathogens to validate the combination strategy of OMT and potentiators.

Table 2. Combination Assay with OMT and Colistin agaisnt *E. coli*

Strain		MIC (μg/mL) of each compound		MIC (μg/mL) of OMT in combination with colistin	
		OMT	Colistin	OMT + colistin[a]	OMT + colistin[b]
E. coli	ARBank#48	>64	0.25	8	64
E. coli	ARBank#55	>64	0.5	4	8
E. coli	ARBank#69	>64	0.5	8	64
E. coli	ARBank#450	>64	0.25	2	64
E. coli	KUB4015	>64	0.5	4	16
E. coli	KUB4019	>64	0.25	8	64
E. coli	KUB4021	>64	0.25	2	16
E. coli	KUB4038	>64	0.25	8	32
E. coli	KUB4045	>64	0.25	4	64
E. coli	ATCC25922	>64	0.5	2	8

[a] 1/2MIC represents the concentration of colistin used in the combination assay, [b] 1/4MIC represents the concentration of colistin used in the combination assay

Table 3. Combination Assay with OMT and Colistin agaisnt *K. pneumoiane*

Strain		MIC (μg/mL) of each compound		MIC (μg/mL) of OMT in combination with colistin	
		OMT	Colistin	OMT + colistin[a]	OMT + colistin[b]
K. pneumoniae	ARBanK#41	>64	0.25	16	>64
K. pneumoniae	ARBanK#49	>64	0.25	32	>64
K. pneumoniae	ARBanK#68	>64	0.25	16	>64
K. pneumoniae	ARBanK#523	>64	8	4	8
K. pneumoniae	ARBanK#524	>64	8	≤1	4
K. pneumoniae	ARBanK#525	>64	32	8	8

[a] 1/2MIC represents the concentration of colistin used in the combination assay, [b] 1/4MIC represents the concentration of colistin used in the combination assay

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Conflict of interest The authors declare no conflict of interest.

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