In Vitro Activity of Nacubactam (OP0595) Alone and in Combination with β-lactams against β-lactamase-producing Enterobacterales Isolated in Japan

Y. Nagira, K. Yamada, H. Okade, N. Senju, Y. Tsutsumi, Y. Tabata, K. Kato. Meiji Seika Pharma Co., Ltd., Yokohama, Japan

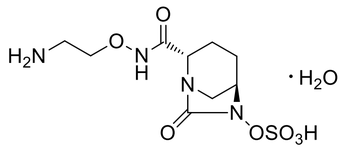
IDWeek 2020
Contact: Kenichiro Kondo, Ph.D.
R&D Planning & Management
Meiji Seika Pharma Co., Ltd.
2-4-16, Kyobashi, Chuo-ku, Tokyo 104-8002, Japan
E-mail: kenichirou.kondou@meiji.com
TEL: +81-3-3273-5498. FAX: +81-3-3273-3439

Abstract

Background: Nacubactam (NAC) is a novel serine β-lactamase inhibitor in clinical development, and inhibits Ambler class A, class C, and some class D β-lactamases. In addition, it has penicillin-binding protein (PBP) 2-dependent antibacterial activity and an 'enhancer' effect when combined with β-lactams bound to PBP3. This study assessed the in vitro activity of NAC alone and in combination with β-lactams against IMP-type metallo-β-lactamase-producing and ESBL-producing Enterobacterales isolated in Japan.
Methods: The MICs for the clinical isolates in Japan were determined and time kill studies were performed. IMP and ESBL genes were detected by PCR. The MICs were determined by broth microdilution method following CLSI methodology. β-lactams and NAC were tested as a ratio of 1:1. Time kill profiles were also studied according to CLSI methodology.
Results: The MIC_{50}/MIC_{90}s of NAC alone against 112 IMP-producing Enterobacterales and 154 ESBL-producing Enterobacterales were 2/>32 and 2/8 mg/L, respectively. Regarding the MICs of cefepime (FEP)/NAC and aztreonam (ATM)/NAC against IMP-producing isolates, the MIC_{90}s were 2 and 1 mg/L and the MIC ranges were 0.06 - 32 and 0.06 - 4 mg/L, respectively. The MIC_{90}s of FEP/NAC and ATM/NAC against ESBL-producing isolates were 0.5 and 1 mg/L. These MIC_{90}s of β-lactam/NAC against IMP-producing and ESBL-producing isolates were significantly lower than those of β-lactam alone (>128 mg/L). The highest MIC of ATM/NAC against IMP-producing isolates was lower than that of FEP/NAC. In addition, bactericidal activities of β-lactam/NAC were observed at the lower concentration of β-lactam compared to that of β-lactam alone.
Conclusions: NAC in combination with β-lactams showed excellent in vitro activities against not only ESBL-producing Enterobacterales but also IMP-producing Enterobacterales isolated in Japan. ATM/NAC tended to show higher antimicrobial effect against IMP-producing isolates by the enzyme stability of ATM. These results support the complex activities of NAC which works as a β-lactamase inhibitor, an antibacterial agent and also an enhancer when combined with β-lactams. Furthermore, these data will be useful for selecting a partner β-lactam for NAC.

Introduction

The production of β-lactamases by gram-negative bacteria remains one of the most significant threats in clinical settings. Nacubactam (OP0595, NAC) discovered by Meiji Seika Pharma Co., Ltd., is a novel serine-β-lactamase inhibitor containing diazabicyclic scaffold.
Nacubactam acts in the three ways:
i) a β-lactamase inhibitor
ii) an antibiotic agent for Enterobacterales
iii) an "enhancer" of activity of various β-lactam agents for Enterobacterales



Methods and Results

Methods

MIC determination
ATCC and NCTC strains obtained from American Type Culture Collection and Public Health England, ESBL-producing Enterobacterales (n=154) and IMP-producing Enterobacterales (n=112) isolated in Japan were investigated. MICs were determined by broth microdilution method following Clinical and Laboratory Standards Institute (CLSI) methodology. Cefepime (FEP), aztreonam (ATM), NAC, meropenem (MEM), imipenem (IPM), piperacillin/tazobactam (TZP), ciprofloxacin (CIP) and amikacin (AMK) were used in this study. β-lactams in combination with NAC were tested as a ratio (1:1) and with fixed 4 mg/L NAC. These were shown by adding (1:1) and (4) after compound name, respectively.
Time-kill study
Time-kill curves of FEP/NAC and ATM/NAC against IMP-producing Enterobacterales were investigated according to the CLSI guideline. Drug concentrations were set based on MIC. A ratio of 1:1 was used as MICs of β-lactams in combination with NAC.

Table 1. Antimicrobial spectrum against Gram-positive and Gram-negative bacteria

Organism, Primary β-lactamase	MIC (mg/L)				
	FEP	FEP /NAC (1:1)	ATM	ATM /NAC (1:1)	NAC
Escherichia coli ATCC 25922	0.06	0.03	0.12	0.12	1
Escherichia coli ATCC 35218, TEM-1	0.03	0.03	0.06	0.06	2
Escherichia coli NCTC 13353, CTX-M-15	>128	0.5	>128	1	2
Escherichia coli ATCC BAA-2469, NDM-1	128	2	16	0.5	2
Klebsiella oxytoca ATCC 13182	0.03	0.06	0.25	0.06	32
Klebsiella pneumoniae ATCC 10031	0.016	0.016	0.06	0.06	2
Klebsiella pneumoniae ATCC 43816	0.03	0.03	0.06	0.06	8
Klebsiella pneumoniae ATCC 700603, SHV-18	1	0.25	64	1	4
Klebsiella pneumoniae ATCC BAA-1705, KPC-2	32	0.5	>128	1	2
Klebsiella pneumoniae ATCC BAA-2814, KPC-3	>128	1	>128	1	2
Klebsiella pneumoniae ATCC BAA-2470, NDM-1	>128	2	>128	2	>128
Klebsiella pneumoniae ATCC BAA-1706	16	1	4	1	>128
Citrobacter freundii ATCC 8090	0.016	0.016	0.03	0.03	4
Enterobacter cloacae ATCC 13047	0.06	0.12	2	0.25	4
Morganella morganii ATCC 25830	≤0.004	≤0.004	≤0.004	≤0.004	16
Proteus mirabilis ATCC 29906	0.06	0.06	0.008	0.008	>128
Proteus vulgaris ATCC 29905	0.03	0.03	0.008	0.008	>128
Serratia marcescens ATCC 13880	0.06	0.06	0.12	0.12	>128
Pseudomonas aeruginosa ATCC 27853	1	2	4	4	64
Acinetobactor baumannii ATCC BAA-2093, OXA-89	2	2	32	16	>128
Acinetobactor baumannii NCTC 13304, OXA-27	32	8	64	32	>128
Burkholderia cepacia ATCC 25416	64	4	128	16	32
Stenotrophomonas maltophilia ATCC 13637	32	2	>128	4	>128
Staphylococcus aureus ATCC 29213	4	2	>128	>128	>128
Enterococcus faecalis ATCC 29212	16	16	>128	>128	>128
Enterococcus faecium ATCC 19434	4	2	>128	>128	>128
Bacillus subtilis ATCC 6633	1	1	>128	128	>128

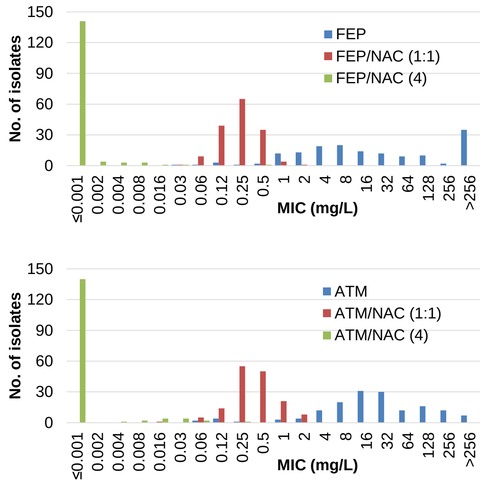


Figure 1. MIC distributions against ESBL-producing Enterobacterales isolated in Japan (n=154)

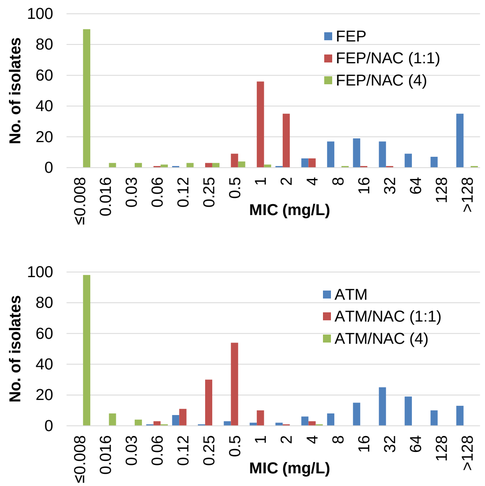


Figure 2. MIC distributions against IMP-producing Enterobacterales isolated in Japan (n=112)

Table 2. Summary of susceptibility testing against ESBL- and IMP-producing Enterobacterales isolated in Japan

	ESBL (n=154)					IMP (n=112)				
Compound	MIC_{50}	MIC_{90}	Range			MIC_{50}	MIC_{90}	Range		
FEP	16	>256	0.03	-	>256	32	>128	0.12	-	>128
FEP/NAC (1:1)	0.25	0.5	0.03	-	2	1	2	0.06	-	32
FEP/NAC (4)	≤0.001	≤0.001	≤0.001	-	0.5	≤0.008	0.12	≤0.008	-	>128
ATM	16	256	0.06	-	>256	32	>128	0.06	-	>128
ATM/NAC (1:1)	0.5	1	0.016	-	2	0.5	1	0.06	-	4
ATM/NAC (4)	≤0.001	≤0.001	≤0.001	-	0.25	≤0.008	0.016	≤0.008	-	4
NAC	2	8	0.25	-	>256	2	>32	1	-	>32
MEM	0.03	0.06	0.004	-	4	16	32	0.03	-	>128
IPM	≤0.12	0.5	≤0.12	-	2	0.5	4	0.12	-	64
TZP (4)	2	16	0.06	-	>256	8	128	1	-	>128
CIP	>16	>16	0.016	-	>16	2	>32	0.008	-	>32
AMK	2	8	0.5	-	64	2	4	0.5	-	>128

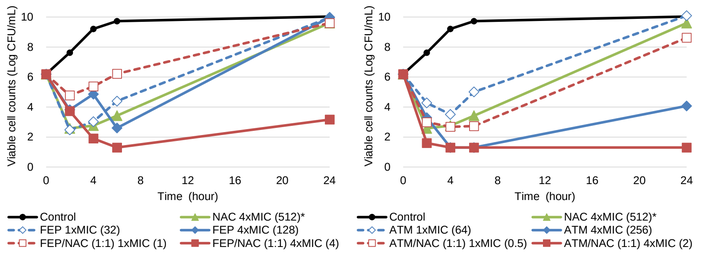


Figure 3. Time-kill curves against IMP-6-producing *K. pneumoniae* MSC 21678
Detection limit was 1.3 log CFU/mL. The drug concentration (mg/L) for each condition is shown in parentheses in the legend. This study was conducted by converting MIC of NAC (>64 mg/L) to 128 mg/L (*).

Conclusion

- NAC showed antimicrobial activity alone against some Enterobacterales and potent combination effect with FEP and ATM against β-lactamase positive isolates.
- NAC in combination with β-lactams showed excellent in vitro activities against not only ESBL-producing Enterobacterales but also IMP-producing Enterobacterales isolated in Japan.
- ATM/NAC tended to show higher antimicrobial effect against IMP-producing isolates by the enzyme stability of ATM.
- These results support the complex activities of NAC which works as a β-lactamase inhibitor, an antibacterial agent and also an enhancer when combined with β-lactams.
- These data can characterize the favorable antimicrobial activities against each β-lactamase producing Enterobacterales depending on the partner β-lactam for NAC.

Acknowledgments

This research was supported by AMED under Grant Number JP18pc0101028.
We thank the following institutions and people for the provision of clinical isolates.
・Laboratory of Bacterial Drug Resistance, Gunma University Graduate School of Medicine
・Study of Bacterial Resistance Kinki Region of Japan
・Hibiki AMR Laboratory, Fukuoka, Japan
・Shizuo Kayama[1-3] and Motoyuki Sugai[1-3] ([1]Antimicrobial Resistance Research Center, National Institute of Infectious Diseases, Tokyo, [2]Hiroshima University Project Research Center for Nosocomial Infectious Diseases and [3]Dept Antimicrobial Resistance, Hiroshima University GSBS)