Investigating the Effect of PAβN to MICs of Ciprofloxacin and Expression of Efflux Pump Genes in *Acinetobacter baumannii* Clinical Isolates

1. Fatma KAYNAK ONURDAG, fatmakaynak@trakya.edu.tr, Trakya University Faculty of Pharmacy Department of Pharmaceutical Microbiology, Edirne, Turkey
2. Suzan OKTEN, suzanokten@trakya.edu.tr, Trakya University Faculty of Pharmacy Department of Pharmaceutical Microbiology, Edirne, Turkey
3. Ugur KAYIS, uurkayis@gmail.com, Aydın University Vocational School of Health Services Department of Pharmacy Services, İstanbul, TURKEY

INTRODUCTION

Acinetobacter spp. are opportunistic pathogenic bacteria causing nosocomial infections. Among them, *Acinetobacter baumannii* is the most important species in terms of the infections caused. *Acinetobacter spp*. can be isolated from a wide variety of infections. In our country, it has been one of the most isolated Gram negative bacilli causing hospital acquired infections.

A. baumannii isolates can develop resistance to antibiotics by different chromosomal and plasmid mediated mechanisms. The resistance mechanism developed by Efflux pump (EFP) systems has been in great importance. The most realistic approach in recent years is researching the inhibition of resistance and enlightening the inhibition mechanisms rather than synthesizing new compounds.

So, it is inevitable to develop compounds that inhibit the effects of EFP systems to prevent EFP-dependent resistance. One of these compounds, phenylalanine-arginine-betanaphthylamide (PAβN), is a dipeptide amine compound. It has been shown that the compound often inhibits the "Resistance Nodulation Division" (RND) type pump systems that cause multiple drug resistance. It is one of the most studied EFP Inhibitor (EPI) which has already been shown to inhibit fluoroquinolone resistance in other bacteria.

In this study, it is aimed to determine i) the effect of PAβN, on MIC of ciprofloxacin, ii) to obtain the ideal inhibitor concentration that eliminates the ciprofloxacin resistance and iii) to obtain the effect of PAβN to overexpression of multi drug efflux pump genes and for these purposes a real time quantitative PCR was designed.

MATERIAL / METHODS

In our study, 70 clinical isolates were collected from Trakya University Health Center for Medical Research and Practice and 68 of them were corrected to be *A. baumannii*. Sixtyseven of the 68 isolates were determined to be resistant to ciprofloxacin and ciprofloxacin susceptibility was investigated for these isolates in presence of PAβN again. Antimicrobial susceptibility testing were done by microdilution method. Thirtytwo isolates determined to have 4 or more fold decrease in ciprofloxacin MIC values were included in checker board assay and q-RT-PCR. FIC indexes and inhibitor concentrations that inhibit ciprofloxacin resistance were calculated according to the checker board assay results. The effect of the combinations were reported as synergic or additive.

RESULTS

As a result; it was considered that checker board assay is sufficient to show the inhibitory effect and to calculate the ideal inhibitory concentrations. However, the effect of the inhibitor to the efflux pump gene expression levels was also investigated with q-RT-PCR. There is a significant difference between groups containing ciprofloxacin alone and ciprofloxacin in combination with PAßN in case of AdeA, AdeB, AdeC, AdeF, AdeG, AdeH, AdeL, AdeR and AdeS genes expression levels ($p<0,05$). There is not a significant difference between groups; medium alone and containing ciprofloxacin in combination with PAßN, except the case of AdeA gene expression levels ($p<0,05$).

Table 1. Checker board assay results of an isolate (ciprofloxacin+PAβN)

	1	2	3	4	5	6	7	8	9	10	11	12
A	0/0	16/0	8/0	4/0	2/0	1/0	0,5/0	0,25/0	0,125/0	0,0625/0	0,03125/0	0,015625/0
B	0/100	16/100	8/100	4/100	2/100	1/100	0,5/100	0,25/100	0,125/100	0,0625/100	0,03125/100	0,015625/100
C	0/50	16/50	8/50	4/50	2/50	1/50	0,5/50	0,25/50	0,125/50	0,0625/50	0,03125/50	0,015625/50
D	0/25	16/25	8/25	4/25	2/25	1/25	0,5/25	0,25/25	0,125/25	0,0625/25	0,03125/25	0,015625/25
E	0/12,5	16/12,5	8/12,5	4/12,5	2/12,5	1/12,5	0,5/12,5	0,25/12,5	0,125/12,5	0,0625/12,5	0,03125/12,5	0,015625/12,5
F	0/6,25	16/6,25	8/6,25	4/6,25	2/6,25	1/6,25	0,5/6,25	0,25/6,25	0,125/6,25	0,0625/6,25	0,03125/6,25	0,015625/6,25
G	0/3,125	16/3,125	8/3,125	4/3,125	2/3,125	1/3,125	0,5/3,125	0,25/3,125	0,125/3,125	0,0625/3,125	0,03125/3,125	0,015625/3,125
H	0/1,5625	16/1,5625	8/1,5625	4/1,5625	2/1,5625	1/1,5625	0,5/1,5625	0,25/1,5625	0,125/1,5625	0,0625/1,5625	0,03125/1,5625	0,015625/1,5625

FIC S = MIC $S_{combination}$ /MIC S
FIC P = MIC $P_{combination}$ /MIC P
FIC = 4/32+ 6.25/25 = 0.125 + 0.25 = 0.375 ≤ 0,5 Synergic Effect

Table 2. Primer sequences, targets and references

Primer target/name	5'-3' DNA Sequence	Reference	Ratio of similarity; mostly similar gene number in GenBank for isolate 1 amplicons
AdeA-461F	CTGAGCCACCACCGGCTAAA	This study	%100; 213155370
AdeA-461R	TTGCCCAATACGCCCAGAAA	This study	
AdeB-351F	TCATGGGTTCAAGCGGTCAA	This study	%100; 213155370
AdeB-351R	CGAGTGGCACAACCAGCATC	This study	
AdeC-171F	AAATGCAGTGCGGCAGGTTAG	This study	%100; 213155370
AdeC-171R	ACAGCCTCTTTCGCGCTTTG	This study	
AdeR-377F	GGTGAAGCCTTTTAACCCAAATGAA	This study	%100; 213155370
AdeR-377R	TATATCCCACGCCACGCACA	This study	
AdeS-120F	GTCACGGCGACCTCTCTGCT	This study	%100; 213155370
AdeS-120R	GCGCATTTTTGACGGAAACCT	This study	
AdeL-346F	CAATCAACCGCTTCTCCAACC	This study	%100; KR297239
AdeL-346R	TGGGTTTACCGCGTGCTTCT	This study	
AdeF-422F	GGTAACGGCGCACAGGTTTT	This study	%100; KR297239
AdeF-422R	TCTTGTTGGGCACCGAGTTTT	This study	
AdeG-143F	TATCGCGGTTTTCCCACCAC	This study	%100; KR297239
AdeG-143R	GGGCTGATTGTGCTGCCTTC	This study	
AdeH-369F	TCTCCGGCTTCACTCGGTTT	This study	%100; KR297239
AdeH-369R	GGCAGTGGTTTGTGCGGTAG	This study	
rRNA-F	AGA GTT TGA TCC TGG CTC AG	Lane 1991	Previously validated primer pairs
rRNA-R	AAG GAG GTG ATC CAG CCG CA	Lane 1991	

CONCLUSION

Since PCR is a laborious and expensive method; it is suggested to use the checker board assay in cases aim to obtain just the inhibitory effect and to use molecular methods to show in which gene expression the inhibitory effect is determined. We come to the conclusion that, the q-RT-PCR method that we designed may be used to manipulate the future researches which will be held by our research team to enlighten the mechanisms of action of the newly synthesized compounds and to associate them with the efflux pump genes expression.