In vitro assessment of the synergy between Polymyxin B (PMB) and Polymyxin B Nonapeptide (PMBN) and Antibiotics on Biofilms from Diabetic Foot Infections

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Background

Increasing resistance of Gram-negative bacteria isolated from nosocomial infections and chronic wounds, such as diabetic foot ulcers has renewed research interests in the use of polymyxins in the treatment of multidrug resistant infections.[1,2] The added resistance conferred by biofilm development in such infections and the absence of novel antibiotics make polymyxins the drugs of choice, in spite of their nephrotoxicity and neurotoxicity.[1,2,5]

Polymyxins are natural non-ribosomal cationic cyclic lipopeptides isolated from the bacterium *Paenibacillus polymyxa*.[4] They exert their antibacterial action by selectively binding to lipid A component of the lipopolysaccharide (LPS) and subsequently disrupt the outer membrane of Gram-negative pathogens.[3,6]

The effects of PMB and PMBN have been previously assessed on planktonic bacteria isolated from various infections.

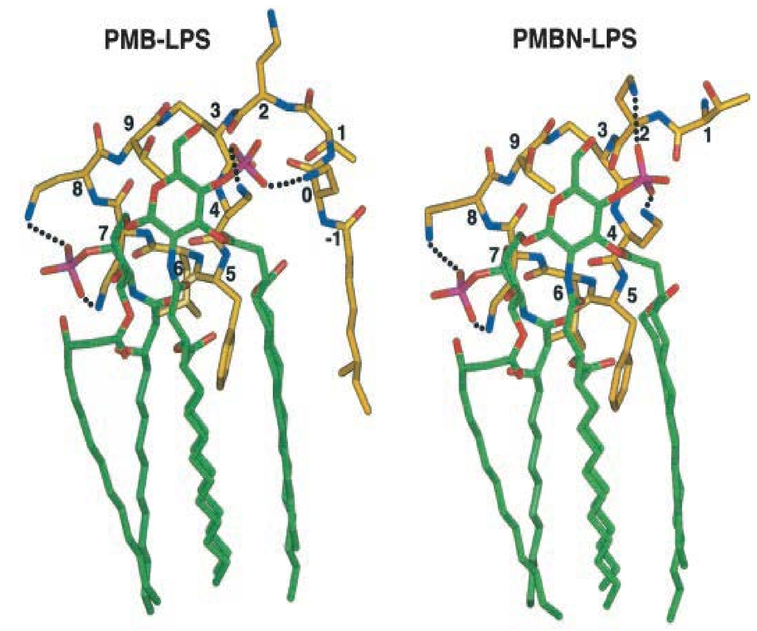

Figure 2. **Molecular modelling of PMB-LPS and PMBN-LPS complexes during cell-membrane disruption (Tsusbery *et al*., 2002).[6]**

Aim of Study

This study assessed the combined effects of cell-membrane permeabilisers (PMB/PMBN) and two antibiotics (ceftazidime (CAZ) and levofloxacin (LEV) in an attempt to develop a strategy for biofilm disruption using the Minimum Biofilm Eradication Concentration Physiology and Genetic assay (MBEC™ P & G, Innovotech Inc, Edmonton, Alberta, Canada)

Methods

The MBEC P&G assay was carried out according to manufacturer's instruction. *Klebsiella pneumoniae* (*K. pneumoniae*) and *Proteus mirabilis* (*P. mirabilis*) biofilms of initial broth suspensions of 10^8 colony forming units per mL were cultivated on the pegs of the MBEC device overnight. They were subsequently challenged with 5120 µg/mL of both ceftazidime and levofloxacin in a ten-fold dilution assay in the presence of 100 and 500 µg/mL PMB and PMBN.

Results

From the tables of results (Tables 1 and 2), it can be deduced that both ceftazidime and levofloxacin are very effective in inhibiting biofilm development (as shown by percentage inhibition (PI%)) when augmented with PMB and PMBN (Figures 1 and 2). This is about 100-fold increase in efficacy when compared to the antibiotics used on their own. The percentage reduction (PR%) in biofilm was also increased considerably when PMB and PMBN concentrations were increased to 500 µg/mL. PMB was more effective than its less antibacterial derivative PMBN. Levofloxacin was also found to be more effective than ceftazidime when combined with both PMB and PMBN due to its enhanced cell-membrane permeability and as an anti-DNA replication uncoupling agent.

Table 1. **MBEC (µg/mL) and MIC (µg/mL) of ceftazidime and levofloxacin against *K. pneumoniae* and *P. mirabilis* in the presence of PMB.**

Organism	Antibiotic	MIC (µg/mL)		MBEC (µg/mL)		PI (%)		PR (%)	
K. pneumoniae	$PMB_{K.p}$*	100	500	100	500	73	90	24	35
	Ceftazidime	39.94	19.97	640	640	90	90	50	>60
	Levofloxacin	39.94	9.98	640	640	>90	>90	>50	>70
P. mirabilis	$PMB_{P.m}$**	100	500	100	500	29	77	10	26
	Ceftazidime	9.98	19.87	640	320	70	>70	50	60
	Levofloxacin	9.98	9.98	640	320	>90	>95	>50	>60

****K.p** – *Klebsiella pneumoniae* ***P.m** – *Proteus mirabilis*

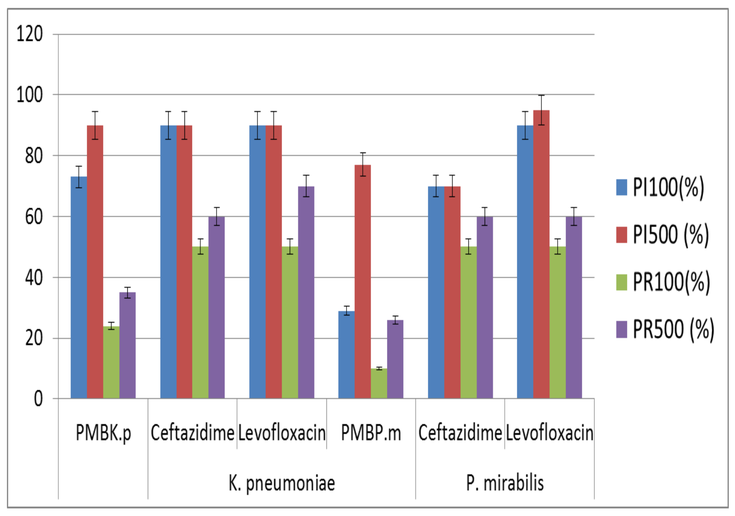

Figure 1. **Percentage inhibition (PI%) and percentage reduction (PR%) of *K. pneumoniae* and *P. mirabilis* biofilms in the presence of 100 and 500 µg/mL $PMB_{K.p}$ only and in combination with ceftazidime and levofloxacin respectively.**

Table 2. **MBEC (µg/mL) and MIC (µg/mL) of ceftazidime and levofloxacin against *K. pneumoniae* and *P. mirabilis* in the presence of PMBN.**

Organism	Antibiotic	MIC (µg/mL)		MBEC (µg/mL)		PI (%)		PR (%)	
K. pneumoniae	$PMBN_{K.p}$	100	500	100	500	60	77	22	30
	Ceftazidime	39.94	19.97	640	640	>70	>80	50	>50
	Levofloxacin	39.94	9.98	640	640	60	95	70	>70
P. mirabilis	$PMBN_{P.m}$	100	500	100	500	25	62	9	25
	Ceftazidime	9.98	19.97	640	320	60	>60	30	>40
	Levofloxacin	9.98	9.98	640	320	>90	>95	50	>60

[a] **(PI) – Percentage (%) inhibition of biofilm at 100 and 500 µgm/mL MIC respectively**
[b] **(PR) – Percentage (%) reduction of biofilm at 100 and 500 µgm/mL MBEC respectively.**

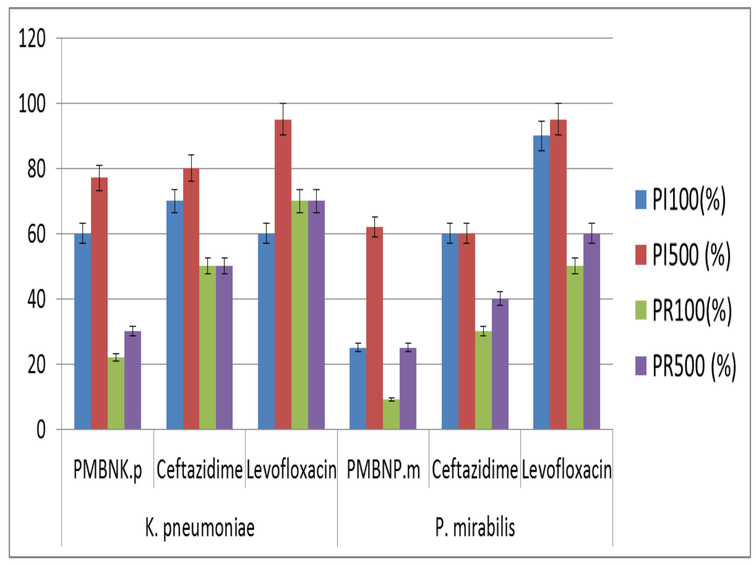

Figure 2. Percentage inhibition (PI%) and percentage reduction (PR%) of *K. pneumoniae* and *P. mirabilis* biofilms in the presence of 100 and 500 µg/mL $PMBN_{K.p}$ only and in combination with ceftazidime and levofloxacin respectively.

Discussion

Though the polymyxins have proven to be the last resort in the treatment of multidrug resistant Gram-negative pathogens, their nephrotoxicity and neurotoxicity have complicated their use.[1,2,3,5,7]

Previous studies have suggested the development of optimal dosage regimen with enhanced efficacy and reduced toxicity.[5] However, this has not been possible as there is scanty knowledge on their pharmacology. Some Gram-negative pathogens have also developed resistance against polymyxins

It has also been predicted that the combination of polymyxins with other antibiotics may reduce their side effects and resistance development.[7]

In this study, the synergy between ceftazidime and levofloxacin and PMB and PMBN have suggested that effective combinations may provide alternate strategies towards biofilm eradication.

Though the effective combinations used in this study yielded about 90% inhibition and 70% reduction in biofilms, they were above their therapy range.

It has been suggested that modulation of the amino acid groups and the hydrophobic regions of PMB and PMBN may increase their efficacy with reduced toxicity.

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