Synergistic effects of synthetic peptides and Carbapenems

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The increasingly frequent isolation of multidrug-resistant *Pseudomonas aeruginosa* (MDRPA) is a major source of clinical concern. The aim of this study was to explore the antimicrobial activity of a synthetic peptide (AMP38) and its synergy with imipenem against imipenem-resistant *P. aeruginosa* infections. AMP38 was manually synthesized following standard Fmoc/tBu procedures using DIPCDI/HOBt activation on Rink amide resin. Once the sequence was assembled, cleavage of the peptide from the resin was carried out by acidolysis with TFA/triisopropylsilane/water (95:3:2, v/v) for 90 min. TFA was removed with a stream nitrogen gas. The oily residue was treated with dry diethyl ether and the precipitated peptide was isolated by centrifugation. The homogeneity of the peptide crude was assessed by analytical HPLC on Nucleosil C18 reverse-phase columns (4x250 mm, 5 µm particle diameter, and 120 Å porous size). Elution was carried out at 1 mL min^{-1} flow with mixtures of H_2O containing 0.045% TFA and acetonitrile containing 0.036% TFA and UV detection at 220 nm. . Cyclization of the peptide was carried out in 5% dimethyl sulfoxide aqueous solution for 24 h and lyophilized twice. The peptide was subsequently purified by preparative HPLC on a Waters DeltaPrep 3000 system with a Phenomenex C18 column (250x10 mm, 5 µm) eluted with H_2O/acetonitrile/0.1% TFA gradient mixtures and UV detection at 220 nm. Final purity was greater >99% according to analytical HPLC. The peptide was characterized by matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectrometry with a PerSeptive Biosystems Voyager-DE instrument. MALDI-TOF MS, m/z ($C_{55}H_{96}N_{16}O_{12}S_2$): 1237.9 $[M+H]^+$, 1259.7 $[M+Na]^+$, 1275.7 $[M+K]^+$, 1219.9 $[M-H_2O]$ a The main mechanism of imipenem resistance in *P. aeruginosa* is the loss or alteration of the outer membrane protein OprD, a porin whose main physiological role remains unknown allowing bacterial penetration by carbapenems. Time-kill and eradication biofilm (MBEC) determinations were carried out using a clinical strain imipenem-resistant. The mechanism of action was examined by transmission electron microscopy. AMP38 resulted to be markedly synergistic with imipenem when determined in imipenem –resistant *P. aeruginosa*. MBEC obtained for the combination of AMP38 and imipenem was of 62.5 µg/ml whereas MBEC of each of antimicrobials separately was 500 µg/ml. AMP-38 should be regarded as a promising anrtimicrobial to figth MDR *P. aeruginosa* infections. Moreover, killing effect and antibiofilm activity of AMP38 plus imipenem was much higher than that of colistin plus imipenem. Perspectives on the use of antimicrobial peptides as door openers will be discussed.