Association of Usnic Acid and Tobramycin as a Therapeutic Strategy against Multidrug-Resistant Escherichia coli and Klebsiella pneumoniae

SARAH LUIZE FIGUEREDO DE ALENCAR PEREIRA [1], PAULA LAIS BORGES DE CARVALHO [1], MARIA DO SOCORRO ALENCAR LIMA [1], AMANDA MONTEIRO LIMA [1], MICHELLY MOREIRA SANTANA [1], MELQUISEDEC DE SOUSA OLIVEIRA [1], MARTON KAIQUE DE ANDRADE CAVALCANTE [1], ALESSANDRA SILVA ARAUJO [2,3], ISABELLA MACÁRIO FERRO CAVALCANTI [3], **LUÍS ANDRÉ DE ALMEIDA CAMPOS** [1,2]

[1.] UNIVERSIDADE DE PERNAMBUCO, CAMPUS OURICURI, OURICURI/PERNAMBUCO, BRASIL
[2.] UNIVERSIDADE DE PERNAMBUCO, FACULDADE DE CIENCIAS MÉDICAS, RECIFE/PERNAMBUCO, BRASIL
[3.] UNIVERSIDADE FEDERAL DE PERNAMBUCO, INSTITUTO KEIZO ASAMI, RECIFE/PERNAMBUCO, BRASIL

Introduction: The increasing spread of multidrug-resistant Enterobacteriales, such as *Escherichia coli* and *Klebsiella pneumoniae*, represents a public health challenge, especially when strains are resistant to conventional antibiotics, reducing pharmacological treatment alternatives. In this context, the search for new therapeutic strategies, including the use of natural compounds such as usnic acid (UA) and its combination with antibiotics like tobramycin (TOB), has been explored with the aim of enhancing antimicrobial activity and promoting the resensitization of resistant strains. **Objective:** This study aimed to investigate the antibacterial activity of usnic acid (UA) and tobramycin (TOB), alone and in combination, against clinical isolates of multidrug-resistant Enterobacteriales. **Materials and Methods:** Isolates of *E. coli and K. pneumoniae* were obtained from the microorganism bank of the Keizo Asami Institute (iLIKA/UFPE). Antibacterial activity was evaluated using the broth microdilution method, according to the Clinical and Laboratory Standards Institute (CLSI) guidelines, to determine the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC). Breakpoints to determine whether the strain is sensitive or resistant to TOB were established using the breakpoint tables for interpreting MICs and halo diameters from the Brazilian Committee on Antimicrobial Susceptibility Testing – BrCAST. The interaction between AU and TOB was analyzed using the Checkerboard method with calculation of the fractional inhibitory concentration index (FICI). **Results:** The results demonstrated high efficacy of tobramycin against the evaluated strains, with MIC values ??of 1 µg/mL for *E. coli* H100407 (susceptible), 2 µg/mL for *E. coli* NCTC 13846 (susceptible), and 4 µg/mL for *K. pneumoniae* ATCC 700603 (resistant) and K A2 29 (resistant), and MBC of 4 µg/mL for *E. coli* H100407, 8 µg/mL for *E. coli* NCTC 13846, *K. pneumoniae* ATCC 700603, and K A2 29. In contrast, usnic acid did not show antibacterial activity at the tested concentrations, with MIC and MBC values ??greater than 1000 µg/mL for all isolates. In vitro interaction analysis between AU and TOB revealed ICIF values ??ranging from 0.6 to 0.18, characterizing synergistic interactions for all strains evaluated with reduction in MIC of approximately 40 times for TOB and 1000 times for AU, evidencing the potentiation of TOB antibacterial activity and a possible resensitization of TOB-resistant strains. **Discussion:** The results suggest that UA may act as a modulator of bacterial resistance, favoring antibiotic action through mechanisms that may include alterations in cell membrane permeability or interference with DNA, altering protein synthesis and metabolic pathways. The significant reduction in the required concentrations of TOB and UA reinforces the potential of the

combination as a strategy to restore the susceptibility of resistant strains and reduce exposure to high doses of antimicrobials. **Conclusion:** Thus, the combination of natural compounds and antibiotics is consolidated as a promising therapeutic approach in addressing bacterial resistance, especially against multidrug-resistant strains of *Escherichia coli and Klebsiella pneumoniae*, and may contribute to expanding therapeutic options. However, further studies, especially in vivo and toxicological evaluations, are necessary to validate safety and clinical applicability.

Agradecimentos: We thank the National Council for Scientific and Technological Development (CNPq) for funding the project and the PIBIC scholarship awarded to Sarah Pereira and Michelly Santana, as well as the Pernambuco State Foundation for Support of Science and Technology (FACEPE) for funding the project and the BIA scholarship awarded to student Amanda Monteiro.