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Brief Original Article

Enhancement of antimicrobial activity of pump inhibitors associating drugs

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Abstract

Introduction: with the continuous emergence of pathogenic resistance to conventional drugs through efflux pumps, increasing efforts are directed toward discovering efflux inhibitory molecules.

Methodology: in this study three P-glycoprotein (P13CP, P22CP, P34CP) efflux-inhibitors (EIs), belonging to the series of phenoxymethylquinoxalines capable to restore/potentiate the antiproliferative activity of doxorubicin and vincristine against human tumor cell lines and different antibiotics against clinical isolates, were investigated on 10 clinical strains of *Candida* and 12 clinical and ATCC strains of Gram positive and Gram-negative bacteria.

Results: MFC values of FLC were reduced in all *Candida* strains by the P22CP and P34CP inhibitors, and in 5/10 fungal strains by the P13CP inhibitor.

Conclusion: novel antibiotics with new modes of action are urgently required to suppress the rise of MDR bacteria. An alternative approach would be to identify molecules that can interfere with the process of efflux.

Key words: Bacteria; efflux; resistance.

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Introduction

The rapid spread of bacteria expressing multidrug resistance (MDR) has necessitated the discovery of new antibacterials and resistance-modifying agents. Various mechanisms provide bacteria with resistance to antibiotics; these include target-site modification, antibiotic inactivation and reduction of cytoplasmic antibiotic concentration. Bacteria can reduce the intracellular accumulation of antibiotics by decreasing their permeability or increasing active efflux of the antibiotic [1]. To become MDR, a bacterium must acquire multiple mechanisms, and while many species have done so, the spectra of resistance vary from case to case. Many efflux pumps, ABC (ATP-binding cassette) transporters, are encoded chromosomally and their presence enhances resistance mediated by these individual mechanisms. Consequently, presence or augmented expression of efflux pumps is responsible for reduced drug availability to inhibit the specific target. With the continuous emergence of pathogenic resistance to conventional drugs through efflux pumps, increasing efforts are directed toward discovering efflux inhibitory molecules. In this study we have used 3 new Quinoxaline derivatives (Qds) as ABC transporter inhibitors (EIs) against several resistant microorganisms. The results of this study showed the importance of EIs in strengthening antibiotic effect against resistant strains.

Methodology

In this study, three P-glycoprotein (P13CP, P22CP, P34CP) efflux-inhibitors (EIs), belonging to the series of phenoxymethylquinoxalines capable to restore/potentiate the antiproliferative activity of doxorubicin and vincristine against human tumor cell lines and different antibiotics against clinical isolates of *M. tuberculosis* and Nontuberculous mycobacteria (NTM) strains [2-3], were investigated on 10 clinical strains of *Candida* and 12 clinical and ATCC strains of Gram positive and Gram-negative bacteria (Table 1). Minimal fungicidal concentration and Minimal bactericidal concentration (MFC, MBC) was determined both for EIs and conventional drugs (fluconazole for *Candida* strains and gentamicin for bacteria isolates). MFC and MBC were defined as the lowest concentration inhibiting 99% of fungal and bacterial growth respectively. Drug potentiation or synergistic action of EIs was assessed evaluating the

values of MFC and MBC when EIs were used in association with antimicrobial compounds. Briefly, fungal and bacterial isolates were grown on Sabouraud-dextrose and Columbia Blood agar respectively and incubated overnight. 100 µL of fungal and bacterial suspensions of a density of 0.5 McFarland were inoculated in 96-well microtiter plates and 100 µL of drugs, EIs and conventional drugs combined with EIs ware added. The plates were incubated at 37°C for 24-48 hours, then 10 µL of microbial suspension was removed, seeded on Sabouraud-dextrose and Columbia Blood agar plates, and incubated for 24-48 hours at 37ºC to determine the MFC and MBC. Positive and negative controls were included in all the experiments. Concentration range was: for fluconazole (FLC) 128-0,01 µg/mL, for gentamicin (GEN) 32-0.0025 µg/mL and for EIs 1000-0,03 µg/mL. The concentration of each EIs to be used in combination with drugs correspond to ¼ of its MFC or MBC to be devoid antimicrobial activity. Cytotoxicity activity of EIs was assessed on alveolar macrophages [4]. Each experiment was performed in duplicate and repeated three times.

Results

The MFC and MBC of the FLC, GEN, EIs and the drug combined with EIs for each of the strains enrolled in this study, are given in Table 1. MFC values of FLC were reduced in all *Candida* strains by the P22CP and P34CP inhibitors, and in 5/10 fungal strains by the P13CP inhibitor. The best activity was found in the strain of *C. krusei*-1 where the MFC value was reduced with all EIs by four-fold and in the strain of *C. krusei-2* where the MFC value was reduced with P22CP and P34CP by four-fold.

Discussion

This result is very interesting considering that *C. krusei* has been recognized as a potentially multidrug-resistant fungal pathogen, due to its intrinsic FLC resistance [5]. The upregulations of multidrug efflux pump were implicated in most FLC-resistant *Candida* strains, for example it has been shown that that transporters of ABC-family are upregulated in azole-resistant isolates of *C. glabrata* with an MFC higher than 16 µg/mL for FLC [6]. In our study the MFC of *C. glabrata* was halved by the action of the EIs confirming

Table 1. Value of MFC and MBC of the FLC, GEN, EIs and the drug plus EIs for microbial strains.

Candida strains	**MFC µg/mL P13CP**	**MFC µg/mL P22CP**	**MFC µg/mL P34CP**	**MFC µg/mL FLC**	**MFC µg/mL P13CP+FLC**	**MFC µg/mL P22CP+FLC**	**MFC µg/mL P34CP+FLC**
C. albicans-1	1000	62,5	500	0.5	0.25	0.25	0.25
C.albicans-2	500	62,5	500	0.5	0.5	0.25	0.25
C. parapsilosis-1	1000	250	1000	2	1	1	1
C. parapsilosis-2	500	250	1000	2	2	1	1
C. tropicalis-1	1000	250	1000	1	0.5	0.5	0.5
C. tropicalis-2	500	250	1000	1	1	0.5	0.5
C. glabrata-1	500	500	500	128	64	64	64
C. glabrata-2	500	1000	500	128	128	64	64
C. krusei-1	1000	500	1000	128	16	16	16
C. krusei-2	500	62,5	1000	64	64	16	16
Bacteria strains	**MBC µg/mL P13CP**	**MBC µg/mL P22CP**	**MBC µg/mL P34CP**	**MBC µg/mL GEN**	**MBC µg/mL P13CP+GEN**	**MBC µg/mL P22CP+GEN**	**MBC µg/mL P34CP+GEN**
Staphylococcus aureus ATCC43330	> 1000	> 1000	> 1000	5	> 0.5	2	2
S. aureus (clinical strain)	> 1000	> 1000	> 1000	1	1	0.5	0.5
Pseudomonas aeruginosa ATCC27853	1000	500	1000	5	2	2	1
P. aeruginosa-1	1000	500	1000	4	2	2	2
P. aeruginosa-2	> 500	500	> 500	2	1	1	1
P. aeruginosa-3	> 500	500	> 500	2	1	1	1
P. aeruginosa-4	> 500	500	> 500	4	2	2	2
Enterobacter aerogenes	> 500	1000	> 500	1	1	0.5	0.5
Proteus mirabilis	> 500	1000	1000	1	1	0.5	1
Citrobacter freundii	> 500	> 500	1000	1	1	0.5	0.5
Providencia stuartii	> 500	> 500	500	4	4	2	2
Acinetobacter baumannii	500	1000	500	> 32	> 32	> 32	> 32

MFC: Minimal Fungicidal Concentration), MBC: Minimal Bactericidal Concentration, FLC: fluconazole, GEN: Gentamicin, EIs: Efflux inhibitors.

the role of efflux pump in the azole-resistance. P22CP and P34CP were able to reduce the MBC of GEN in 11/12 and 10/12 bacteria strains respectively and P13CP in 5/12 (Table 1). *P. aeruginosa ATCC27853* was the strain for which was found the best activity of EIs with a MBC reduction of 2.5 fold determined by P13CP and P22CP, and of five-fold by P34CP. It's interesting underline that susceptibility to gentamicin was restored. The cytotoxicity assay indicated that the P13CP and P34CP EIs were not cytotoxic by concentrations ≤ 250 μg/mL, instead P22CP was not cytotoxic for concentrations ≤ 125 μg/mL. The results obtained in this study indicated that the efflux activity contributes to the overall resistance in microbial strains, and that the inhibition of efflux pumps by the EIs can enhance the clinical effect of antibiotics that are their substrates.

Conclusion

The problems of resistant Gram-positive and Gram-negative bacteria highlight the urgent need for new drugs with new modes of action and/or combination therapy to treat infections caused by resistant human pathogens such as *S. aureus*, *Pseudomonas aeruginosa* and *Candida spp*.. Novel antibiotics with new modes of action are urgently required to suppress the rise of MDR bacteria. An alternative approach would be to identify molecules that can interfere with the process of efflux.

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References

1. Piddock LJ (2006) Multidrug-resistance efflux pumps - not just for resistance. Nat Rev Microbiol 4: 629-636.
2. Carta A, Loriga M, Piras S, Paglietti G, La Colla P, Busonara B, Collu G, Loddo R (2006) Synthesis of variously substituted 3-phenoxymethyl quinoxalin-2- ones and quinoxalines capable to potentiate *in vitro* the antiproliferative activity of anticancer drugs in multi-drug resistant cell lines. Med Chem 2: 113-122.
3. Carta A, Piras S, Paglietti G, Pricl S, La Colla P, Busonara B, Loddo R(2008) 2(3)-Aryl-thio(oxy)-methylquinoxaline derivatives: A new class of P-glycoprotein-mediated drug efflux inhibitors. Med Chem 4: 194-205.
4. Cannas S, Usai D, Pinna A, Benvenuti S, Tardugno R, Donadu M, Zanetti S, Kaliamurthy J, Molicotti P (2015) Essential oils in ocular pathology: an experimental study. J Infect Dev Ctries 9: 650-654. doi: 10.3855/jidc.6842.
5. Fukuoka T, Johnston DA, Winslow CA, De Groot MJ, Burt C, Hitchcock CA, Filler SG (2003) Genetic basis for differential activities of fluconazole and voriconazole against *Candida krusei*. Antimicrob Agents Chemother 47: 1213-1219.
6. Silva LV, Sanguinetti M, Vandeputte P, Torelli R, Rochat B, Sanglard D (2013) Milbemycins: more than efflux inhibitors for fungal pathogens. Antimicrob Agents Chemother.57: 873-886.

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