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Review

Plant phenolics and terpenoids as adjuvants of antibacterial and antifungal drugs

Susana A. Zacchino[a,*], Estefania Butassi[a], Melina Di Liberto[a], Marcela Raimondi[b], Agustina Postigo[a], Maximiliano Sortino[a,c]

[a] Área Farmacognosia, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, Rosario 2000, Argentina
[b] Area Microbiología, Facultad de Cs. Médicas, Universidad Nacional de Rosario, Santa Fe 3100, Rosario 2000, Argentina
[c] Área Micología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, Rosario 2000, Argentina

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ABSTRACT

Background: The intensive use of antibacterial and antifungal drugs has dramatically increased the microbial resistance and has led to a higher number of difficult-to-eradicate infections. Combination therapy with two or more antimicrobial drugs has emerged some years ago to overcome the issue, but it has proven to be not completely effective. Natural secondary metabolites of MW ≤ 500 represent promising adjuvants for antimicrobials and have been the object of several researches that have increased in the last two decades.
Purpose: The purpose of this Review is to do a literature search of the natural compounds that showed high enhancing capacity of antibacterials' and antifungals' effects against planktonic bacteria and fungi and to analyze which are the natural products most used in combination with a focus on polyphenols and terpenoids.
Results: One hundred of papers were collected for reviewing. Fifty six (56) of them deal with combinations of low MW natural products with antibacterial drugs against planktonic bacteria and forty four (44) on natural products with antifungal drugs against planktonic fungi. Of the antibacterial adjuvants, 41 (73%) were either polyphenols (27; 48%) or terpenes (14; 25%). The remaining 15 papers (27%), deal with different class of natural products. Since most natural potentiators belong to the terpene or phenolic structural types, a more detailed description of the works dealing with these type of compounds is provided here. Bacterial and fungal resistance mechanisms, the modes of action of the main classes of antibacterial and antifungal drugs and the methodologies most used to assess the type of interactions in the combinations were included in the Review too.
Conclusions and perspectives: Several promising results on the potentiation effects of antifungals' and antibacterials' activities by low MW natural products mainly on polyphenols and terpenes were reported in the literature and, in spite of that most works included only *in vitro* assays, this knowledge opens a wide range of possibilities for the combination antimicrobial therapy. Further research including *in vivo* assays and clinical trials are required to determine the relevance of these antimicrobial enhancers in the clinical area and should be the focus of future studies in order to develop new antimicrobial combination agents that overpass the drawbacks of the existing antibiotics and antifungals in clinical use.

Abbreviations: ABC, ATP-Binding Cassette; AMPH, Amphotericin B; AMC, Amoxicillin/Clavulanic acid; AMP, ampicillin; CAZ, ceftazidine; CD, clerodane diterpene 16α-hydroxyclerodа-3,13(14)-*Z*-dien-15,16-olide; CDR, *Candida* drug-resistance; CEP, cephapirin; CFU, Colonies forming units; CIP, Ciprofloxacin; CLSI, Clinical Laboratory Standards Institute; CPM, Carbapenem; CUR, Curcumin; DAP, Daptomycin; DRI, Dose Reduction Index; ECZ, Econazole; EGCg, Epigallocatechingallate; ECV, epidemiologic cut-off values; EPI, Efflux Pump Inhibitors; ERY, Erythromycin; ERSA, Erythromycin-resistant *Staphylococcus aureus*; EUCAST, European Committee on Antimicrobial Susceptibility Testing; FIC, Fractional Inhibitory Concentration; FICI, Fractional Inhibitory Concentration Index; FCZ, Fluconazole; ILSMR, Intensifiers Specifically of β-Lactam against Methicillin-resistant *Staphylococcus aureus*; IMP, Imipenem; ITZ, Itraconazole; KTZ, Ketoconazole; LVX, Levofloxacin; LZD, Linezolid; MCZ, Miconazole; MDR, Multidrug resistant; MFS, Major facilitator superfamily; MIC, Minimum Inhibitory Concentration; MPM, Meropenem; MRSA, Methicillin-Resistant *Staphylococcus aureus*; MSSA, Methicillin Sensitive *Staphylococcus aureus*; MW, Molecular weight; Nor, Norfloxacin; Nys, Nystatin; OFL, Ofloxacin; OXA, Oxacillin; OXY, Oxytetracicline; PPM, Panipenem; PCZ, Posaconazole; PBP, Penicillin Binding Protein; PEN, Penicillin; PG, propyl gallate; PRSP, Penicillin resistant *Streptococcus pneumoniae*; PIP, Piperacillin; PPM, Panipenem; QUI, Quinolone; RNA, Ribonucleic acid; Sulb, Sulbactame; Str, Streptomycin; Terb, Terbinafine; TET, Tetracyclin; TRSA, Tetracyclin-resistant *Staphylococcus aureus*; VA, Vancomycin; VCZ, Voriconazole; VRE, Vancomycin-resistant *Enterococcus spp*; VRSA, Vancomycin-resistant *Staphylococcus aureus*

* Corresponding author.
E-mail address: szaabgil@citynet.net.ar (S.A. Zacchino).

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Introduction

The intensive use of antibacterial and antifungal drugs has dramatically increased the frequency of microbial resistance (Andersson and Diarmaid, 2010) and has led to an increase of difficult-to-eradicate infections. To overcome the issue, combination therapy with two or more antimicrobial drugs has emerged some years ago (Cuenca-Estrella, 2004) in the belief that they can achieve a reversal of microbial resistance with lower quantities of each substance and can also lower the known antimicrobial drugs' toxic side-effects (Lewis and Kontoyiannis, 2001). In spite of the many advantages of combination therapy, several reports have proven that it has failed in several patients (Kristiansen et al., 2007) possibly due to the efficacy relies largely on the results of the *in vitro* studies and experimental animal models and evidences from well-designed clinical trials are lacking (Cuenca-Estrella, 2004).

In the last years, the testing of combinations of antimicrobial drugs with non-antimicrobial compounds (therapeutic agents not originally designed for this purpose) appears to be a new promising strategy to cope with treatment failures (Bush et al., 2011; Ejim et al., 2011; Lehtinen and Lilius, 2007). As an example, Afeltra et al. (2004) reported the *in vitro* positive interactions between itraconazole (ITZ) and seven different non-antimicrobial membrane-active compounds against ITZ-susceptible and ITZ-resistant *Aspergillus fumigatus* strains.

Among the non-antimicrobial compounds, natural metabolites of MW ≤ 500 may represent promising adjuvants of antimicrobials' effects (Hemaiswarya et al., 2008; Langeveld et al., 2014).

According to previous reports (Wagner and Ulrich Merzenich, 2009) the potentiation of the antimicrobial activity by a natural product can be achieved by different mechanisms such as (i) multi-target effect, in which each compound targets a different site in the microbial cell; (ii) pharmacokinetic or physicochemical effects (*i.e.* improvement of solubility or bioavailability of the antimicrobial drug); (iii) targeting a specific resistance mechanism of microorganisms that is the major challenge of the combination therapy.

In this Review, we have made a literature search in order to have a look into the natural low MW metabolites that have shown enhancing microbial growth inhibition capacity of antibacterials (antibiotics) and antifungals against bacterial and fungal planktonic cells. Of them, a detailed analysis of the terpenoid or phenolic structures is provided. Previously, the most used methodologies to assess the antimicrobial effects of compounds alone or in combination was added to the Results section in order to a better comprehension of the results.

In addition, the main classes of antibacterial and antifungal drugs and their targets, the mechanisms of resistance for each type of drugs were included with the aim of facilitating the understanding on how the combination of an antibacterial or antifungal drug with a low MW natural product can work.

Materials and methods

Search strategy

The search for suitable papers was performed in Internet databases (PubMed, Sciencedirect and other web pages, by using the following keywords: "bacterial infections", "fungal infections" "planktonic cells", "secondary metabolites", "enhance", "enhancers", "synergism", "natural products", "potentiators" ; "antifungal drugs", "antibacterial drugs", "chemosensitizing agents", "*in vitro*", "*in vivo*" . Additional papers were included in our collection after surveying the references from the selected articles. We explored articles that use *in vitro* and *in vivo* experimental systems.

Data extraction

The information gathered from the chosen articles included: the structures of natural potentiators; the concentrations at which they act as enhancers; the fungal or bacterial strains used; the *in vitro* and *in vivo* assays and the assessments of molecular mechanisms of the antimicrobial effects of the combinations. The information was divided into two groups: (a) Natural products in combination with antibacterial drugs against bacterial planktonic cells; (b) Natural products in combination with antifungal drugs against fungal planktonic cells.

Results and discussion

Methodologies most used to assess the type of interactions in the combinations

The analysis of adjuvancy in most of the reviewed works were carried out *in vitro* by using the microdilution assay in the checkerboard design which allows the calculation of the Fractional Inhibitory Concentration (FIC) of each partner and the Fractional Inhibitory Concentration Index (FICI) values for the combinations (see Supplementary material). In some of the works, isobolograms and time-kill studies (Berembaum, 1989; Martínez Irujo et al., 1996; Sun et al., 2008; White et al., 1996) were also performed. It is worth to take into account that only few studies performed *in vivo* studies and the studies of the mechanism of action of the mixtures are scarce (Ballar and Coote, 2016; Campbell et al., 2012; Gupta et al., 2016; Han, 2007).

The Dose Reduction Index (DRI) (Chou, 2006, 2010), a measure on how many times the MIC of the antimicrobial drug is reduced by its partner when tested in combination (MIC antimicrobial alone/MIC antimicrobial in the mixture) was included in this Review when it was possible. A greater DRI for an antimicrobial drug is indicative of a greater adjuvant capacity for a given effect level.

Modes of action of the main classes of antibacterial drugs

There are four proven targets for the main antibacterial drugs: (1) bacterial wall biosynthesis; (2) bacterial protein synthesis; (3) bacterial DNA replication and repair and (4) bacterial RNA synthesis (ECDC/EMEA, 2009; Kohanski et al., 2010, Moore, 2013; Walsh, 2000). Most structural types that act for each mechanism of action are detailed in Table 1.

Antibacterial combinations

Bacterial resistance and its mechanisms

The resistance of a bacterium to a given antibiotic is assessed by determining the MIC of the antimicrobial substances against the microorganisms. This information, together with the known pharmacokinetic properties of the substance, allows the characterization of the bacteria as "susceptible", "intermediate" or "resistant" to a given antibiotic (Rodloff et al., 2008). The testing techniques for MIC determination must be standardized to make the test results reproducible, because parameters such as the culture medium, inoculum size, incubating temperature and time, all influence the results. The Clinical Laboratory Standards Institute (CLSI) of the United States and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) of the European Union have established guidelines that allow the harmonisation of antimicrobial breakpoints throughout the world and define breakpoints for new agents (Brown et al., 2015).

The bacterial resistance can be classified as clinical and microbiological and, in turn, it can be primary (intrinsic) or secondary (acquired). Bacteria can show intrinsic resistance as a result of its own structural characteristics (Blair et al., 2015) (Fig. 1) or can also acquire it *via* mutations of chromosomal genes and by horizontal gene transfer (Andersson and Hughes, 2009; Sandegren and Andersson, 2009).

In general the bacterial resistance can be mediated by several mechanisms that fall into three main groups: (a) those that minimize the intracellular concentrations of the antibiotic as a result of efflux or poor