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Resistance Reversals in *Candida spp.* A Vision from Drugs, Plant Derivatives and Membrane Transporters

Resistencia reversa en *Candida spp.* Una visión desde fármacos, derivados de plantas y transportadores de membrana

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Abstract

Worldwide, infectious diseases caused by filamentous fungi and yeasts are becoming more frequent due to their adaptation to drugs and different resistance mechanisms. There are around fifteen *Candida* species that affect humans, with high morbidity and mortality rates; one of its most studied resistance mechanisms is the drug efflux pump, in which the ABC and MFS transporters energize the transport of substances through ATP and proton gradient, respectively. The use of resistance reverters could be considered an objective of study instead of creating new antifungals. The effectiveness of antifungals can be increased by combining them with natural substances. In this review, we address issues such as the classification of antifungal drugs, general mechanisms of resistance, ABC and MFS transporters, use of plant derivatives to reverse resistance processes, and laboratory evaluation of potential reverters, derived from plants.

Keywords: resistance reversals, ABC and MFS transporters, membrane transporters, plant derivatives.

Resumen

Mundialmente, las infecciones generadas por hongos filamentosos y levaduras, están volviéndose más frecuentes debido a su adaptación a fármacos y a diferentes mecanismos de resistencia. Las especies de *Candida* que afectan a los humanos son alrededor de quince, con tasas elevadas de morbimortalidad; uno de sus mecanismos de resistencia más estudiados es la bomba de eflujo de fármacos, en la que los transportadores ABC y MFS energizan el transporte de sustancias mediante ATP y gradiente de protones, respectivamente. El uso de revertidores de la resistencia, podría considerarse un objetivo de estudio en lugar de crear nuevos antifúngicos. La eficacia de los antifúngicos puede aumentar mediante combinación con sustancias naturales. En esta revisión, se abordan cuestiones como la clasificación de los fármacos antifúngicos, mecanismos generales de resistencia, transportadores ABC y MFS, uso de derivados de plantas para revertir procesos de resistencia y la evaluación de laboratorio de los posibles revertidores derivados de plantas.

Palabras clave: resistencia reversa, transportadores ABC y MFS, transportadores de membrana, derivados de plantas.

Introduction

Infectious diseases are an important problem for public health, in addition to the high costs involved in controlling them. New resistant microorganisms are currently emerging, which worries organizations worldwide, turning this resistance issue into a study objective. These infectious diseases are a significant source of death, with almost 50,000 deaths per day (1, 2). The mortality rate caused by some fungi is much higher than Gram-negative bacteria, which leads to consider the study of fungi in the field of infectious diseases in humans (3). Between 2013 and 2017, the incidence of candidemia was approximately 9 per 100,000 people, and approximately 25,000 cases occur in the United States each year (4). Recent global estimates have found many infections by multiple fungi, among which approximately 700,000 cases of invasive candidiasis have been reported (5).

Because of these high health impacts and high costs to health entities, new antifungals with activity against resistant fungi are urgently required. However, it must be considered that fungi are eukaryotic, just like human cells; therefore, it is challenging to discover new antifungal agents that do not interfere with our cells (6).

Compounds that are clinically successful against eukaryotic pathogens have had to exploit unique characteristics, limiting the search for compounds that generate successful drugs to combat these pathogens (7). Generally, it takes almost a decade for an antifungal to be approved for clinical use (8). Fungi develop different resistance mechanisms to survive the unfavorable environment provided by antifungals. One of these vital mechanisms is the release of ABC and MFS transporters, which function as drug efflux pumps (9). For this reason, inhibitors of the fungal efflux pump can be taken into consideration since they could represent compounds of great importance for the development of drugs and reverse the resistance (10).

The use of chemical substances different from the current antifungal drugs, in combination with antifungal drugs, can be used to improve the therapy's effectiveness and reverse the resistance processes in clinical strains. Attempts have been made to address treatment failures, combining different antifungals, or combining antifungals with non-antifungals (11, 12). The application of drug combinations has been recognized as an essential area since 1979 by Bennett *et al.* (13). Today, more attempts have been made to address treatment failures by combining different in vitro and in vivo antifungals (14, 15).

One way to overcome drug resistance is to obtain chemical or natural substances that make fungi sensitive to the effects of antifungals, potentiating their efficacy (16).

Several substances have been combined with antifungals to reverse the resistance processes. In this review, we name some: tacrolimus (FK506) (12, 17, 18), ibuprofen (19), licofelone (11), histatin-5 (20), extract of Phialocephala fortinii (21), sulfa drugs (22), diorcinol D (14) and budesonide (23).

As previously discussed, many papers show the synergism of substances not derived from plants that reverse the resistance to fungal drugs; however, few articles highlight the use of plant derivatives with this synergism.

For the development of this document, a search was carried out in Pubmed (the searcher of biomedical and life sciences literature) for publications associated with membrane transporters ABC and MFS,

plant extracts, and natural products associated with drug resistance (fluconazole). Any article that used chemicals and synthetics related to resistance reversal was excluded.

Classification of antifungal drugs

The constant evolution of medicine has led to new treatments and therapies that help patients with a wide variety of diseases and patients undergoing clinical or surgical procedures that need medication to control and/or prevent infections. Currently, there are a lot of immune or infectious events that generate immunosuppression, which leads to a high frequency of fungal infections.

In the 1950s, amphotericin B, a powerful drug in the fight against fungi, was developed. However, its side effects at the kidney and liver level remained a significant problem for treating invasive fungal infections. Later came the development of azoles, which have many action targets without side effects (24, 25).

Not only is the development of the drug important, but so is the route of administration since some medications have very good absorption at the gastric level, and others are only available at the parenteral level (25).

Antifungal drugs can be organized in different ways. This review details two interesting classifications developed by different investigation groups in consecutive years. Nett, Andes and collaborators (2016) organized antifungal drugs according to their targets, spectrum, pharmacology and resistance processes shown by fungi. They proposed four groups: polyenes, flucytosine, echinocandins, and azoles; in the latter, a wide range of azole drugs are described (25).

Another quite complete classification was carried out by Campoy and Adrio's group in 2017, in which they described seven groups: inhibitors of ergosterol biosynthesis, fungal membrane disruptors, drugs that act in the synthesis of the fungal cell wall, drugs that act in the biosynthesis of sphingolipids, inhibitors of nucleic acid synthesis, inhibitors of protein biosynthesis and inhibitors of microtubule biosynthesis. This classification describes the different drugs for clinical use, their form of action, their targets and their main characteristics. Azoles such as fluconazole, polyenes such as amphotericin B, and echinocandins such as caspofungin are among the most commonly mentioned drugs (26).

All these routes or mechanisms of action of the mentioned drugs have their effect against different types of fungi. However, the route of administration, the proper use of these, the patient's conditions, and the fungus's characteristics can lead to resistance, aggravating the patient's conditions, increasing their hospital stay, and generating public health problems with high costs for the patient and health entities.

Resistance to antifungal drugs

One way to evaluate antifungal resistance to a specific drug is to examine the growth of fungal strains in vitro in the presence of different drug concentrations. The most used way to measure

this activity is through dilutions in liquid media (27). Two protocols are used worldwide principally (CLSI, Clinical Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing), which settle standards and guidelines for accurate antifungal susceptibility testing. Clinical and research entities use these protocols to determine if a specific strain is resistant to a particular drug, establishing minimum inhibitory concentration values (MIC), which are used to express the fungus sensitivity to the compound being tested (28).

Microbiological resistance is defined by the presence of a resistance mechanism to the drug being tested, directly depending on the microorganism. It can be classified as primary or innate when the fungi are resistant before exposure to the drug or secondary or acquired which appears in response to exposure to the drug (6, 29).

General mechanisms of antifungal resistance to commonly used drugs

There are different mechanisms by which fungi can resist other drugs, and these can be organized directly in relation to the drug's group used, such as azoles or polyenes, or through a more generalized and complete classification, such as the one performed by Dominique Sanglard, which includes three categories: (1) decrease in the effective concentration of the drug, (2) alterations of the pharmacological objective, and (3) metabolic derivations (28). In relation to the decrease in the effective concentration of the drug, it describes four different mechanisms, and one of the best known is described as the active output flow of the drug through two membrane transport systems: cassette transporters of binding to ATP (ABC) and transporters of the main facilitating superfamily (MFS) (8, 28).

Abc transporters

The ABC proteins in fungi utilize the hydrolysis of ATP to energize the transport of a wide variety of compounds through biological membranes (30, 31).

Researchers have identified 28 putative members of the ABC protein family in *C. albicans* (32, 33). Based on phylogenetic analysis, ABC proteins have been grouped into 6 major subfamilies of transporters (30, 33), among which three of the most studied are related to resistance to pleiotropic drugs (PDR), multidrug resistance (MDR), and protein subfamilies associated with multidrug resistance (MRP). Among these, the most important fungal subfamily often associated with resistance to fungal drugs is the PDR family, with a prominent example being Pdr5p in S. cerevisiae (30, 31, 33). Seven of these ABC proteins have confirmed resistance to multiple drugs, including CDR1, 2, 3, 4, 5, SNQ2, and YOR1 (33).

In *Candida spp*, four members of the PDR subfamily have been characterized. Cdr1p and Cdr2p are involved in drug transport and the translocation of phospholipids, while Cdr3p and Cdr4p are not drug transporters (34). As mentioned, members of ABC superfamilies confer multidrug resistance. In C. albicans, only Cdr1 and Cdr2 have well-documented roles in clinical resistance to medications (34, 35). When discussing ABC proteins in C. glabrata, it should be noted that around 18 ABC transporters have been identified (36), of which CgCdr1, CgCdr2, and CgSnq2 confer phenotypes resistant to multiple drugs (30, 37).

MFS transporters

MFS transporters belong to the largest group of secondary active transporters and function as uniporters, symporters, or antiporters (38). In yeasts, the MFS-MDR transporters operate by proton and are classified into two groups: drug/H+ antiporter-1 family DHA1, and drug/H+ antiporter-2 family DHA2 (31, 39). In C. albicans, 95 putative MFS proteins, grouped into 17 families, have been identified (40). Mdr1 represents a main multidrug transporter with an important role in azole resistance (38, 40). In C. glabrata, more than ten members of the MFS proteins of the DHA1/2 class can be recognized. However, more studies are needed to demonstrate their respective functions (41, 42).

Use of plant derivate to reverse resistance processes

Medicinal plants have been used as treatment for different health problems, both infectious and non-infectious, showing good biological activity in vitro and in vivo, using either their extracts or oils derived from them (43-45). The research of natural products active against *Candida spp*. has increased significantly (46). A review in 2017 collected 111 documents (between the years 1969 and 2015), and there were 142 on anti-*C. albicans* natural products. Therefore, few studies use these extracts to evaluate the synergism with azoles, polyenes or echinocandins for the treatment against infections by *Candida* species (47).

In 2022, Florimar *et al*., evaluated 137 extracts obtained from Argentinian plants, finding that 15 plant extracts overcame the fluconazole resistance of *Candida albicans* and had fungal activity. The extracts from *A. communis* and *S. atripicifolium,* provide antifungal effects by blocking the efflux pums function of Mdr1 and Cdr1 transporters (48).

Due to poor clinical practices with the indiscriminate use of antifungals and the large number of fungi that are achieving resistance, the control of those problems is becoming difficult. For this reason, a good alternative is the use of natural substances, which, when combined with antifungal drugs, can improve effectiveness (49). The total number of plant species reported worldwide is greater than 250,000 and many of these are used for therapeutic purposes (1).

Despite many investigations about the use of plants for medicine, the great majority are used as antifungals directly, using their different parts and extracting a great variety of extracts. Compared to the studies associated with the search for new antifungals, there is little research on using these extracts to reverse resistance processes. Next, investigations about the use of plants as revertants of resistance to antifungal drugs are detailed below.

Sharifzadeh *et al.* (50) tested the in vitro interaction of the combination between thymol—a component of essential oils of plants and fruit skin—and fluconazole against clinical isolates of *Candida*, demonstrating a significant synergistic effect to inhibit the growth of the tested strains. In 2016, Machado *et al*. (51) used the *Acca sellowiana* plant, native to South America, in subtropical climate regions such as Brazil, and analyzed several fractions of the extract of this plant, suggesting that the combination of the active fraction of F2 and fluconazole could be used as an alternative treatment for mucocutaneous infections caused by resistance to *C. glabrata*. Several studies carried out with different plants demonstrated the synergy of using antimycotics that act against *C. albicans* (47, 52). In 2017, researchers used green tea catechins and demonstrated a

synergistic effect with antifungals against bacterial and fungal pathogenic species (53). Cui *et al.* (54) synthesized some derivatives of flavones and isoflavones and showed that some compounds have excellent fluconazole resistance reversal activity against *C. albicans*. One year after, a study with phytocompounds showed a synergism with fluconazole against *Candida* species, leading to a reversal of resistance and showed the interactions of phytocompounds with fluconazole (55). In an interaction study between fluconazole and baicalein against fluconazole-resistant clinical isolates of C. albicans, the researchers showed intense antifungal activity against many of the fluconazole-resistant isolates analyzed (56). Sharma *et al.* (57) showed how curcumin, a natural product, in synergy with antifungals, could reverse the resistance mediated by ABC pumps in yeast. In the same year, another group demonstrated the synergism of fluconazole and plagiochin E isolated from Marchantia polymorpha L. plant, decreasing ergosterol formation by interfering in its pathway of biosynthesis (58).

Many publications show the antifungal effect of plant derivatives, but few investigations are made with plant derivatives to show synergism with antifungal drugs, especially fluconazole. Nevertheless, we show a table with some investigations that used plant derivatives.

Table 1. MIC and FICI range of plant's derivatives in Candida spp. strains

Plants	**Plant derivatives**	**MIC range for plant's derivatives *Candida spp*. strains**	**FICI range Combination Plant derivates/FZL**	**Interaction**	**Reference**
Coptis chinensis Hydrastis canadenses Berberis vulgaris	Berberine	1 – 16 µg/mL	NA	NA	(59)
Thymus vulgaris, Origanum vulgare and Tangerine peel	Thymol	43.7 – 87.5 µg/mL	0.36 - 0.49	Synergy	(50, 60)
			0.56 - 0.60	Additive effect	
Acca sellowiana	Aqueous extract (F2 fraction)	62.5 – 500 µg/mL	0.25 - 0.50	Synergy	(61)
			0.63 - 0.75	Additive effect	
Camellia sinensis	Green tea catechins	125 – 500 µg/mL	0.039	Synergy	(53)
			0.83 - 0.93	Additive effect	
			1.67 - 2.47	Indifference	
NA	Derivatives of flavones and isoflavones	1 - 128 µg/mL	0.25	Synergy	(54)
Scutellaria baicalensis	Baicalein	128 µg/mL	0.037 - 0.098	Synergy	(56)

Marchantia polymorpha	Plagiochin E	16 µg/mL	NA	NA	(58)
C. cyminum and L. binaludensis	Essential oils	3.90 - 11.71 µg/mL	NA	NA	(45)
Sclerocarya birrea, Sterculia setigera	Methanolic extract	3.125 - 50 mg/mL	NA	NA	(44)
A. aethiopicus, C. colocynthis, S. alexandrina, B. juncea, C. longa, C. citratus, G. pillansii and K. delagoensis methanolic extracts	Methanolic extracts	0.15 - 0.81 mg/mL	NA	NA	(43)
Achillea millefolium, Anthemis nobilis, Artemisia annua, Baccharis trimera, Cymbopogon winterianus, Cyperus articulatus, Cyperus rotundus, Lippia alba, Mentha arvensis var. piperita Mentha piperita, Mentha pulegium, Mikania laevigata, Piper aduncum, Plectranthus barbatus, Stachytarpheta cayennensis and Thymus vulgaris.	Oils and extracts	0.25 - 2 mg/mL	NA	NA	(46)
E. uniflora	hydroalcoholic extracts	62.5 - 250 µg/mL	0.31	Synergy	(52)
P. guajava, P. diospyrifolium, R. officinalis, S spectabilis (flowers), S spectabilis (leaves), T. riparia			NA	NA	
P. hispidum			0.37	Synergy	

Synergy, FICI ≤ 0.5; additive effect, FICI > 0.5 - 1.0; indifference, FICI > 1.0 - 4.0; antagonism, FICI > 4.0. FICI: fractional inhibitory concentration index. NA: not applicable. FZL: fluconazole.

Source: Own elaboration.

Conclusion

Candida infections are taking high importance in public health. *C. albicans, C. glabrata, C. parapsilosis, C. krusei* and *C. tropicalis* are the most frequent. Likewise, *C. auris* is one of the emerging yeasts currently, with multiple resistances to various drugs. Given the increased resistance to existing drugs, in this review, we want to show the importance of investigating plant derivatives' synergistic effects to reverse resistance processes to current medicines, especially fluconazole, and thus guarantee effective treatments with low adverse effects.

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Disclosure of Interest

We declare that we have no competing interests.

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