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Review

Combination of fluconazole with non-antifungal agents: A promising approach to cope with resistant *Candida albicans* infections and insight into new antifungal agent discovery

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ABSTRACT

The past decades have witnessed a dramatic increase in invasive fungal infections, especially candidiasis. Despite the development of more effective new antifungal agents, fluconazole (FLC) is still widely used in the clinic because of its efficacy and low toxicity. However, as the number of patients treated with FLC has increased, FLC-resistant *Candida albicans* isolates emerge more frequently. In addition, biofilm-associated infections are commonly encountered and their resistance poses a great challenge to antifungal treatment. Various approaches have been proposed to increase the susceptibility of *C. albicans* to FLC in order to cope with treatment failures, among which is the combination of FLC with different classes of non-antifungal agents such as antibacterials, calcineurin inhibitors, heat shock protein 90 inhibitors, calcium homeostasis regulators and traditional Chinese medicine drugs. Interestingly, many of these combinations showed synergistic effects against *C. albicans*, especially resistant strains. The main mechanisms of these synergistic effects appear to be increasing the permeability of the membrane, reducing the efflux of antifungal drugs, interfering with intracellular ion homeostasis, inhibiting the activity of proteins and enzymes required for fungal survival, and inhibiting biofilm formation. These modes of action and the antifungal mechanisms of various compounds referenced in this paper highlight the idea that the reversal of fungal resistance can be achieved through various mechanisms. Studies examining drug interactions will hopefully provide new approaches against antifungal drug resistance as well as insight into antifungal agent discovery.

1. Introduction

Invasive fungal infections have increased significantly in recent years, coincident with the rising number of immunocompromised patients, the wide development of organ transplantation, tracheal intubation and endoscopic techniques, and the extensive application of broad-spectrum antibiotics, immunosuppressants and corticosteroids [1,2]. *Candida* spp., especially *Candida albicans*, account for a large proportion of invasive fungal infections [3–6]. Although the ratio of non-*albicans Candida* spp. isolated in candidiasis has risen over the past several years, *C. albicans* is still the most common pathogenic fungus [7–9] accounting for 42.5% of infections, followed by *Candida tropicalis* (27.3%), *Candida parapsilosis* (21.9%), *Candida glabrata* (4.4%) and other non-*albicans Candida* spp. [8]. Azoles, especially fluconazole (FLC), have been extensively used in clinical practice because of their great efficacy and reduced toxicity [9,10]. However, with frequent exposure to FLC, FLC-resistant *C. albicans* isolates have emerged [2,7]. In addition, biofilms formed on indwelling catheters, tracheal tubes and endoscopes act as a barrier to the diffusion of antifungal agents, complicating the use of FLC as a single-drug treatment option [11]. Moreover, the development of new antifungal agents in clinical therapy has lagged behind the increasing incidence of drug resistance, and few effective antifungal agents are available.

In recent years, efforts have been made to overcome the emergence of resistant fungi by using drug combinations. However, high costs and serious side effects have put limitations on the combinations of antifungal drugs [12,13]. In addition, contradictory results of either synergistic or antagonistic actions of various antifungal combinations have been reported [10,14,15]. Therefore, the focus of research has shifted to examine the combination of antifungals with non-antifungals. The synergistic mechanisms of drug interactions against resistant isolates can occur in two ways, by overcoming fungal resistance and by enhancing antifungal

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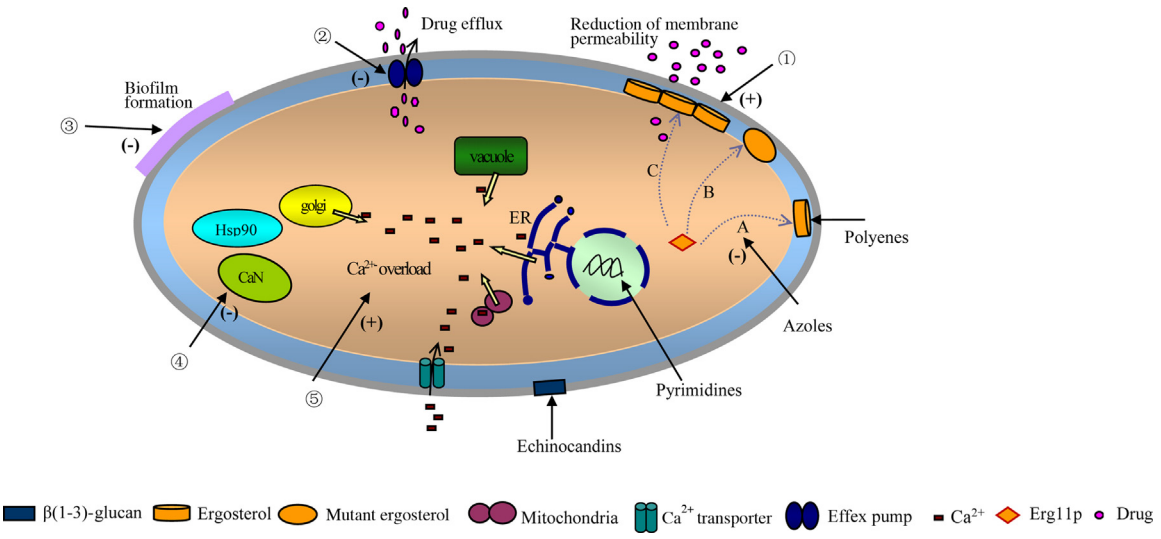

Fig. 1. Illustration of the main antifungal targets, fungal resistance mechanisms and antifungal resistance approaches. A–C represent the ergosterol synthesis pathway and related mechanisms: (A) normal erg11p gene; (B) mutant erg11p gene; and (C) overexpressed erg11p gene. 1–5 represent the antifungal resistance approaches of drugs alone or in combination: (1) increasing membrane permeability; (2) inhibition of drug efflux; (3) inhibition of biofilm formation; (4) inhibition of calcineurin (CaN) and heat shock protein 90 (Hsp90); and (5) disruption of calcium homeostasis. ER, endoplasmic reticulum.

activity. The approaches detailed include: (i) inhibition of sterol biosynthesis, thus increasing membrane permeability [16,17]; (ii) reducing drug efflux, especially by inhibiting expression of efflux pump genes [18,19]; (iii) inhibition of biofilm formation [20–22]; (iv) inhibition of calcineurin and heat shock protein 90 (Hsp90) [23,24]; and (v) disruption of cytoplasmic ionic homeostasis [25–28] (Fig. 1). Here we mainly review the research on FLC in combination with non-antifungal agents such as antibacterials, calcineurin inhibitors, Hsp90 inhibitors, calcium homeostasis regulators, traditional Chinese medicinal drugs and other agents against planktonic *C. albicans* as well as against biofilms. The key findings of several studies indicating synergistic effects of drugs in combination with FLC are summarised in Table 1.

2. Drug interactions of fluconazole in combination with non-antifungal agents

2.1. Fluconazole in combination with antibacterial agents

Deep fungal infections are often accompanied by bacterial infections in patients receiving broad-spectrum antibiotics or immunosuppressive therapy for an extended period of time, leading to an increased chance of antifungals being used in combination with antimicrobials. Therefore, identifying antibacterial agents that can increase the effectiveness of antifungals, especially FLC, has become very important and has garnered wide attention. Here we will discuss the antibacterials found to be synergistic with FLC.

2.1.1. Tetracyclines

Tetracyclines are antimicrobial drugs with a broad spectrum of antibiotic activity, and some of the tetracycline derivatives have been reported to have slight efficacy against *Candida* spp. [29–31]. For example, Schierholz et al. [29] found that the minimum inhibitory concentration (MIC) of minocycline (MNC) for *Candida* spp. was 128–256 μg/mL after 10 days of incubation. Wilson et al. [30] reported an MIC of 256–512 μg/mL after 18 h of incubation at 37 °C, whilst de Oliveira et al. [31] found MICs for *Candida* spp. between 0.25 μg/mL and 1000 μg/mL after 24 h of incubation at 37 °C. These studies all show a high MIC of MNC for *Candida* spp., although the MIC varied in the different studies. However, when MNC was used in combination with FLC against FLC-resistant *C. albicans*, a significant synergistic effect was observed [32], with a reduction of FLC MICs from 512 μg/mL to 2 μg/mL and MNC MICs from 512 μg/mL to 32 μg/mL, demonstrating that MNC significantly increases the susceptibility of the fungus to FLC. Moreover, doxycycline (DOX) [25,33] was also found to be synergistic with FLC, potentiating the antifungal activity of FLC both against sensitive and resistant *C. albicans* in a dose-dependent manner at low concentration (25 μg/mL).

Mechanistic studies demonstrate that the synergy of MNC with FLC against FLC-resistant *C. albicans* was related to enhancement of the ability of FLC to penetrate biofilms as well as a loss of Ca^{2+} homeostasis [32]. DOX-mediated fungal growth inhibition could be reversed by the addition of iron, indicating that DOX synergises with FLC via interference with iron homeostasis [25].

Many manifestations of candidiasis are related to the formation of biofilms on host tissue. These biofilms act as a barrier to the diffusion of antifungal agents and limit the ability of drugs to reach organisms deep in the biofilm [34]. Susceptibility studies have revealed that biofilms formed by *C. albicans* may be up to 1000 times more resistant to antifungal drugs than planktonic cells [35,36] and they become the primary cause of clinical catheter-related infections. Antimicrobial lock therapy has been proposed as an alternative strategy for the prevention of catheter-related bloodstream infections [37,38]. Miceli et al. [20] found that DOX (128 μg/mL) alone had similar activity to FLC (2–1024 μg/mL) against *C. albicans* biofilms, only generating a 22.9% reduction of biofilm metabolic activity. However, when DOX (128 μg/mL) was used in combination with FLC it displayed a significant synergistic effect, with a 58.3% growth reduction. Moreover, when DOX was used alone at a higher concentration (2048 μg/mL) it demonstrated up to an 85% reduction in metabolic activity of the *C. albicans* biofilm. These findings suggest that a high-dose DOX-based antimicrobial lock therapy strategy in combination with traditional antifungal agents may be useful for the treatment of *C. albicans* biofilms. The synergistic effects and mechanisms of FLC in combination with a lower concentration of DOX (1–64 μg/mL) against *C. albicans* biofilms were observed in another study [33]. A significant synergistic effect of the FLC/DOX combination against almost all *C. albicans* biofilms that formed over three time intervals (4, 8 and 12 h) was manifest as a reduction of the sessile MICs of FLC from 64–512 μg/mL to 1–16 μg/mL when in combination with DOX.