Antimicrobial Effectiveness of Maleimides on Fungal Strains Isolated from Onychomycosis

Carla Wanderley Gayoso¹, Edeltrudes de Oliveira Lima², Evandro Leite de Souza²*, Valdir Cechinel Filho³, Vinicius Nogueira Trajano², Fillipe de Oliveira Pereira² and Igara Oliveira Lima³

¹Laboratório de Tecnologia Farmacêutica; Departamento de Ciências Farmacêuticas; Centro de Ciências da Saúde; Universidade Federal da Paraíba; João Pessoa - PB - Brasil.
²Laboratório de Micologia; Departamento de Ciências Farmacêuticas; Centro de Ciências da Saúde; Universidade Federal da Paraíba; João Pessoa - PB - Brasil.
³Núcleo de Investigações Químico Farmacêuticas; Universidade do Vale do Itajaí; Itajaí - SC - Brasil

ABSTRACT

This study aimed to analyze the effectiveness of maleimides as inhibitors on the growth of fungal strains isolated from onychomycosis by the solid medium diffusion procedure. The results showed a promising antifungal activity of the assayed maleimides with formation of fungal growth inhibition halos oscillating between diameter 10 and 23mm. MIC was 100µg/mL for 3,4-dichloro-N-phenyl-methyl-maleimide and 3,4-dichloro-N-phenyl-propyl-maleimide and 200µg/mL for 3,4-dichloro-N-phenyl-ethyl-maleimide, 3,4-dichloro-N-phenyl-buthyl-maleimide, 3,4-dichloro-N-phenyl-maleimide.

Key words: Onychomycosis, fungi, maleimides, antifungal activity

INTRODUCTION

Onychomycosis is a kind of fungal infections in which nails are affected (Sampaio and Rivitti, 1999), more frequently feet nails representing 80% of the cases (Achten and Wanet-Rouard, 1998). Etiologically, approximately 90% of the onychomycosis is caused by dermatophyte fungi such as Tricophyton rubrum, T. mentagrophytes var. interdigitale and Epidermophyton flocosum (Graybill, 1992). Yeasts, mainly Candida genera and moulds (e.g. Scytalidium, Acremonium and Cephalosporium) also have been reported as potential onychomycosis agents causing (Juan and Virendra, 2000).

It has been reported that the use of commonly available pharmaceutical preparations applied in onychomycosis treatment have been some times inefficient (Midgley and Moore, 1996), needing to a search for antifungal compounds which would be more effective, cheaper, with wide antifungal spectrum, short time of use and minimum side effects (Juan and Virendra, 2000).

Maleimides are obtained easily and have presented satisfactory profile to the development of new and efficient drugs (Aquino et al., 2003). These have been characterized as similar to the filantimide alkaloid obtained from Phylantus sellowianus of Euphorbiaceae family (Andricopulo et al., 1998). Some compounds inserted in maleimides group showed expressive pharmacological properties as analgesic, anti-spasmodic, antibacterial and antifungal (Cechinel Filho et al., 1994; Santos et al., 1994; Cruz et al., 1996; Corrêa et al., 1997).
The aim of this study was to evaluate the effectiveness of some maleimides on the growth of fungal strains isolated from onychomycosis.

MATERIALS AND METHODS

Maleimides

Antifungical action of 3,4-dichloro-N-phenyl-maleimide, 3,4-dichloro-N-phenyl-methyl-maleimide, 3,4-dichloro-N-phenyl-ethyl-maleimide, 3,4-dichloro-N-phenyl-propyl-maleimide and 3,4-dichloro-N-phenyl-buthyl-maleimide was analyzed at concentrations of 200, 100, 50, 25, 12.5, 6.3 and 3.1µg/mL. The solutions were prepared in sterile distillated water prior to assays. Molecular structures of these are shown in Fig. 1.

Fungal strains

Candida albicans, C. tropicalis, C. krusei, Tricophyton rubrum, T. mentagrophytes and Geotrichum candidum strains were used as test microorganisms and obtained from clinical samples collected from patients infected with onychomycosis. Their isolation and identification were carried out according to standard procedures (Rebel and Taplin, 1974; Van-Rij, 1984; Hoog and Guarro, 1995).

Antifungical assay

Solid medium diffusion procedure using wells in dishes was devised to evaluate the antifungical activity (Hadaceck and Greger, 2000). Fungal inoculum of approximately 10⁶ CFU/mL standardized by McFarland Scale 0.5 tube and incubation time of 48 hours at 37°C for yeasts and 10-14 days/28°C for moulds were used (Pempel et al., 1986). At the end of the incubation time, the fungal growth inhibition halos diameters were measured in millimeters using calipers. Minimum Inhibitory Concentration - MIC was considered the smallest maleimide concentration able to develop fungal growth inhibition halo equal or higher than 10mm diameter (Lima et al., 1993; Lima et al., 1999). Control assays were performed with ketoconazole (50µg/mL). All assays were performed twice and the results were expressed as average.

RESULTS AND DISCUSSION

Infections caused by fungi including those of dermatological interest have shown increasing occurrence since 1980’s, which has led to search for alternative substances with efficient antifungal properties and little or no toxicity to host (Graybill, 1992; Hazen, 1995). This high occurrence has been inducing factor for studies regarding the antifungical effectiveness of different chemical compounds on dermatological infections etiological agents (Aquino et al., 2003). The results regarding the sensitivity of fungal strains isolated from onychomycosis to maleimides are shown in Table 1. All assayed fungal strains were sensitive to maleimides, 3,4-dichloro-N-phenyl-methyl-maleimide and 3,4-dichloro-N-phenyl-propyl-maleimide which showed the smallest MICs. MIC was 200µg/mL for 3,4-dichloro-N-phenyl-maleimide and 3,4-dichloro-N-phenyl-ethyl-maleimide and 400µg/mL for 3,4-dichloro-N-phenyl-propyl-maleimide and 3,4-dichloro-N-phenyl-buthyl-maleimide. Fungal growth inhibition halos diameter were between 10mm (G. candidum x 3,4-dichloro-N-phenyl-methyl-maleimide, G. candidum x 3,4-dichloro-N-phenyl-ethyl-maleimide, G. candidum x 3,4-dichloro-N-phenyl-buthyl-maleimide) and 23mm (T. mentagrophytes x 3,4-dichloro-N-phenyl-buthyl-maleimide).

Resistant behavior in C. tropicalis and T. mentagrophytes to the standard antifungal (Ketoconazole, 50µg/mL) applied as positive control was observed. T. mentagrophytes was the most sensitivity strain with inhibition halos average diameter of 17.2mm. G. candidum was the least sensitive strain showing inhibition halos average diameter of 10.8mm.

Lima et al. (1999) found prominent antimicrobial activity in imidic compounds which were effective to inhibit the growth of Escherichia coli, Staphylococcus aureus, Candida albicans, Microsporum canis and Penicillium. Dantas et al. (2000) evaluated the sensitivity of Candida species and dermatophytes to maleimides, naftalimides and succimides and noted that only maleimides showed promising results as Candida inhibitor, while the inhibition of Microsporum and Tricophyton was similar for the three assayed compounds.
Cechinel Filho et al. (1994) and Cruz et al. (1996) carried out study regarding the antibacterial activity of cyclic amides, including N-phenylmaleimides and N-aryl-dichloro-maleimides on *S. aureus, Salmonella tiphymurium* and *E. coli* and reported that only maleimides were able to inhibit these microorganisms presenting MIC between 200 and 50µg/mL. Studies regarding the molecular structure - biological activity relationship of maleimides have showed that their antimicrobial activity possibly could be related with the double bound in the imidic ring or with the nitrogen atom next to the benzene ring, which could lead to electronic interactions between maleimides and microbial cells (Cechinel Filho and Yunes, 1998; Dantas et al., 2000). Moreover, increasing electronic density on nitrogen atom suggests participation in the activation of redox cycles that play major role for development of biological activities (Andricopulo et al., 1998).

The results obtained in our study showed prominent effectiveness of maleimides in inhibiting the growth of onychomycosis etiological agents. These findings and the emergent necessity of developing new and efficient antifungical compounds could lead to a thought regarding the
possible rational inclusion of these compounds in pharmaceutical compositions used for the antifungal activity and antifungal activity of N-arylmaleimides: structure activity relationships. 
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RESUMO
Este estudo objetivou analisar a efetividade de maleimidas como inibidores do crescimento de cepas fúngicas isoladas de onicomícoses através da técnica de difusão em meio sólido. Os resultados mostraram destacável atividade antifúngica das maleimidas ensaiadas com a formação de halos de inibição do crescimento fúngico com diâmetros oscilando entre 10 e 23mm. A CIM encontrada foi 100µg/mL para 3,4-dicloro-N-fenil-metil-maleimida e 200µg/mL para 3,4-dicloro-N-fenil-propil-maleimida e 200µg/mL para 3,4-dicloro-N-fenil-maleimida, 3,4-dicloro-N-fenilet-maleimida e 3,4-dicloro-N-fenil-butil-maleimida.

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