Structural alterations in plant compound treated Trichophyton tonsurans

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Citation

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Abstract

The ultra structural changes were studied in Trichophyton tonsurans –a clinical isolate, treated with compound ({- (2-hydroxy-2-methylpropyl)- - [2-hydroxy-3-methylbut-2-en-1-yl] polymethylene}), isolated from ethyl acetate leaf extract of Caesalpinia bonducella. Compound microscopy studies reveal; on normal SDA medium, the germ tubes grew rapidly; long and regularly branched, and their tips appeared normal. But on SDA medium containing 100, 200 and 300 g/ml of compound, spherical hyphae or bamboo-like-joint hyphae occurred after 7 days of incubation. Hyphae developing from the abnormally swollen microconidia were spherical or ellipsoid and shorter and thicker than those developing from normal microconidia. Though all the three concentrations of compound caused morphological changes in Trichophyton tonsurans, the most prominent change seen in T. tonsurans treated with 300 g/ml of compound was characterized by several findings: (i) irregular hyphae with broken cell wall, which was the result of longer exposure time; wavy or shrunken hyphae; (ii) extrusion of cellular materials. However in the control, the hyphae were regular and branched with normal tips.

INTRODUCTION

Fungi comprise one of the five major kingdoms of organisms characterized by a unique specialized chitinous cell wall (Deason, 1997). These eukaryotic nonmotile organisms afford a diverse range of clinical manifestations including allergy, toxic reactions and infections (mycoses) in human and animals (Ajello and Hay, 1998). In recent years, a remarkable increase has been reported in the incidence of different mycoses due to aggressive cancer chemotherapy, widespread use of broad-spectrum antibiotics, increasing in the number of immunosuppressive diseases and highly effective immunosuppressants for organ transplantation (Anaissie et al., 2003). Because of huge similarities between fungal and mammalian cells, there is a limited selective target for designing new antifungal formulations (Barrett, 2002; Georgopapadakou and Walsh, 1994). On the other hand, available drugs, especially polyenes and azoles, suffer from a number of limitations, which can cause some difficulties in their applications. In this regard, host toxicity, drug resistance, drug-drug interactions, fungistatic mode of actions, and limited routes of applications were found to be considered in the treatment with antifungal agents (Georgopapadakou et al., 1996). There is thus an urgent need for new antifungals with new modes of action, broad fungicidal spectrum of action and fewer doses–limited side effects (Graybill, 1996; Maertens and Boogaerts; 2000). In recent years, there is a rapid growth of interest in understanding the mode of actions of plants with known antimicrobial properties, which led to find out new effective compounds like peptide and proteins including low molecular weight compounds.

In this context, there are no reports documented on the morphological changes of fungi grown in presence of {- (2-hydroxy-2-methylpropyl)- - [2-hydroxy-3-methylbut-2-en-1-yl] polymethylene}. This study was undertaken to study the morphological changes in Trichophyton tonsurans under restraint with the compound in order to find out its action on the T. tonsurans.

MATERIALS AND METHODS

COMPOUND MICROSCOPY

The square samples (10 by 10 mm) of T. tonsurans grown in presence of compound at concentrations 100, 200 and 300 g/ml were cut off from plates and mounted in lactophenol cotton blue and examined microscopically for morphological changes.

SCANNING ELECTRON MICROSCOPY

Fungal material obtained from cultures grown either in presence of compound or in absence was processed for

morphological studies at Indian Institute of Science, Bangalore, Karnataka, India.

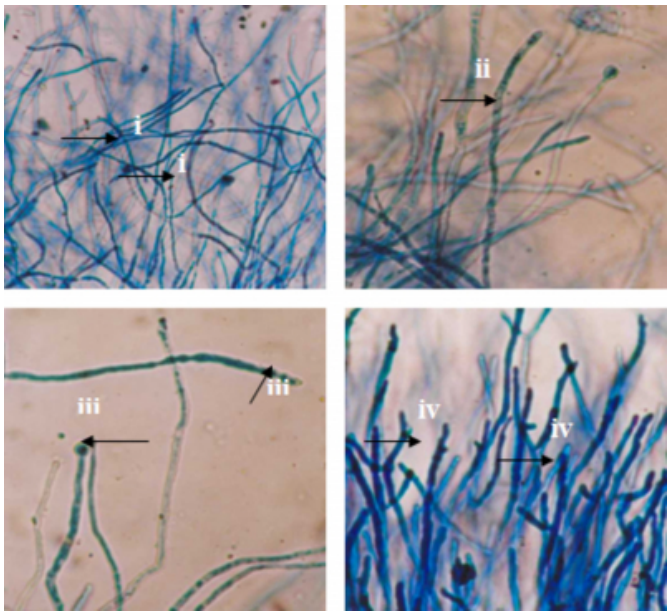
Mycelia samples were recovered from 15 days old cultures. The pathologic changes of compound isolated from the ethyl acetate leaf extract of Caesalpinia bonducella- treated mycelia were analyzed after comparing with control groups grown in absence of the compound.

EXPERIMENTAL RESULTS

Morphological effect of compound on hyphae of T. tonsurans was observed as follows; on normal SDA medium, the germ tubes grew rapidly; long and regularly branched, and their tips appeared normal. But on SDA medium containing 100, 200 and 300 g/ml of compound, spherical hyphae or bamboo-like-joint hyphae occurred after 7 days of incubation (Plate-I). Hyphae developing from the abnormally swollen microconidia were spherical or ellipsoid and shorter and thicker than those developing from normal microconidia.

Figure 1

Plate-I: Photomicrograph of treated with compound.

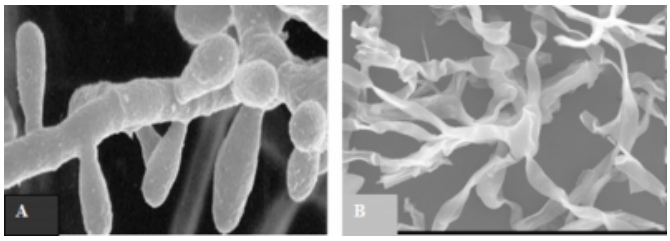


i = long and regularly branched hyphae (Control).
ii= abnormally swollen microconidia, spherical or ellipsoid (Treated with 100 µg/ml)
iii= spherical hyphae or bamboo-like-joint hyphae (Treated with 200 µg/ml)
iv= Shorter, blunt and thicker hyphae (Treated with 300 µg/ml

Microscopic observation on the effect of compound on hyphae of T. tonsurans was performed by scanning electron microscopy study (Plate-II). All the three concentrations of compound caused morphological changes in Trichophyton tonsurans. The most prominent change seen in T. tonsurans treated with 300 g/ml of compound was characterized by several findings: (i) irregular hyphae with broken cell wall, which was the result of longer exposure time; wavy or shrunken hyphae; (ii) extrusion of cellular materials. However in the control, the hyphae were regular and branched with normal tips.

Figure 2

Plate-II: Scanning electron microscopy study of after exposure to 300 µg/ml of -(2-hydroxy-2-methylpropyl)- -[2-hydroxy-3- methylbut-2-en-1-yl] polymethylene for 4 days.



A = control culture which were exposed to DMSO for 4 days showing normal hyphae.
B = treated cultures which were exposed to the compound showing irregular, wavy and shrunken hyphae with excretion of cytoplasmic materials.

DISCUSSION

Along with the increasing number of immunocompromised patients at risk for fungal infections, during the past last decade, there is growing demand for safe and effective antifungal agents. Plant derived compounds are of interest in treating the widespread occurrence of dermal infections caused by dermatophytes. They comprise safer or even more effective substitutes than synthetically produced antimicrobial agents.

In this investigation, the compound {- (2-hydroxy-2-methylpropyl)- - [2-hydroxy-3-methylbut-2-en-1-yl] polymethylene} isolated from ethyl acetate leaf extract of C. bonducella has strongly affected the hyphae of T. tonsurans at 100, 200 and 300 g/ml, particularly at 300 g/ml. The spherical, shorter and thicker hyphae reveal the decomposition of compound in the hyphal region of T. tonsurans.

The scanning electron microscopy results strongly suggest that inhibitory concentrations of the compound affected the normal growth and induced degenerative changes of the hyphae of T. tonsurans probably by affecting some essential

metabolism or structure of the fungal cell. These dramatic changes in hyphae may be due to a severe damage in the fungal cell coat, or perhaps some alteration in the membrane permeability, resulting in the loss of cytoplasm or by severe depletion of hyphal contents. Ultrastructural findings for compound treated fungus showed that it primarily targets the hyphal cell membrane and damages took place by breaking down of the cell wall and degradation of cell organelles. Totally, it seemed that -(2-hydroxy-2-methylpropyl)- - [2-hydroxy-3-methyl but-2-en-1-yl] polymethylene (compound) has caused plasmolysis and finally death of the cell.

Trichophyton tonsurans is an anthropophilic fungus with a world wide distribution causes inflammatory or chronic non-inflammatory finely scaling lesion of skin, nails and scalp. This investigation is a contribution to the area of antifungal chemotherapy, which can be used in the cure of tinea corporis caused by T. tonsurans. But, before the use of this compound, clinical trials should be made in order to prove its antidermatophyte potential.

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