BRAZILIAN JOURNAL OF MICROBIOLOGY

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Medical Microbiology

Anti-fungal potential of ozone against some dermatophytes

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ARTICLE INFO

Article history:
Received 30 June 2015
Accepted 14 October 2015
Available online 21 April 2016
Associate Editor: Karen Spadari Ferreira

Keywords:
Dermatophytes
Dermatophytosis
Ozone
Hydrolytic enzymes
Ozonized oil

ABSTRACT

Dermatophytes are classified in three genera, *Epidermophyton*, *Microsporum* and *Trichophyton*. They have the capacity to invade keratinized tissue to produce a cutaneous infection known as dermatophytoses. This investigation was performed to study the effect of gaseous ozone and ozonized oil on three specific properties of six different dermatophytes. These properties included sporulation, mycelia leakage of sugar and nutrients and the activity of their hydrolytic enzymes. Generally, ozonized oil was found to be more efficacious than gaseous ozone. *Microsporum gypseum* and *Microsporum canis* were the most susceptible, while *Trichophyton interdigitale* and *T. mentagrophytes* were relatively resistant. The study revealed a steady decline in spore production of *M. gypseum* and *M. canis* on application of ozonated oil. An increase in leakage of electrolytes and sugar was noticed after treatment with ozonized oil in the case of *M. gypseum*, *M. canis*, *T. interdigitale*, *T. mentagrophytes* and *T. rubrum*. The results also revealed loss in urease, amylase, alkaline phosphatase, lipase and keratinase enzyme producing capacity of the investigated fungi.

Introduction

Dermatophytosis constitutes an important public health problem, not only in underdeveloped countries but also in elderly and immuno-compromised patients worldwide.[1,2]

The treatment with systemic antifungal chemical agents such as ketoconazole, fluonazole and itraconazole derivatives have side effects, in particular, when these chemicals are used for longterm. Therefore, the search for suitable alternatives to these drugs has been going on. One possible approach is to use ozone therapy. The ozone gas molecule has powerful

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http://dx.doi.org/10.1016/j.bjm.2016.04.014

anti-microbial, germicide properties against viruses, bacteria, parasites and fungi. The interaction of ozone molecule with the oxidizable molecules of cellular components particularly those containing double bonds, sulfhydryl groups, and phenolic rings leads to an oxidation reaction that stunts their growth. Hence, membrane phospholipids, intracellular enzymes, and genomic materials are targeted by ozone. These reactions result in cell damage and death of microorganisms.[3,4]

The cell wall of fungi is multilayered and composed of approximately 80% carbohydrates and 20% of proteins and glycoproteins. The presence of many disulfide bonds making this a possible site for oxidative inactivation by ozone. Ozone has the capacity to diffuse through the fungal wall, enters into its cytoplasm and disrupting vital cellular functions. The inhibitory effect of ozone on spore germination, spore production and biomass production in two *Aspergillus* species was examined by Antony-Babu and Singleton.[5]

The reaction of ozone with olive oil occurs almost exclusively with the carbon–carbon double bonds present in unsaturated fatty acids producing different toxic products such as several oxygenated compounds, ozonides, aldehydes and peroxides. These compounds could be also responsible for the wide antimicrobial activity of ozonized olive oil. The safety of oleozone was reported by Gundarova et al. and Alvarez et al.[6–9]

The aim of this investigation was to study the effect of ozone on the spore germination of various dermatophytes. Since their pathogenecity depends on the activity of keratinolytic and other hydrolysing enzymes, it was important to test the effect of ozone on the production and activity of keratinase, phosphatase, urease, amylase and lipase.

Materials and methods

Test organisms

Five dermatophyte species (*Microsporum canis*, *M. gypseum*, *Trichophyton rubrum*, *T. mentagrophytes*, and *T. interdigitales*) used in this study were obtained from medical laboratory of microbiology at Kasr elainy hospital and identified by routine mycological procedures. The fungi were separately inoculated into fresh plates of Sabouraud dextrose agar (SDA) "for ozone gas exposure" and into fresh slants of SDA "for ozonized oil treatment", then incubated for 3 weeks at 28 °C. From the culture slants, the spore suspensions were prepared to be a working suspension of (8×10^4 conidia/ml). This suspension was used for ozonized oil treatment.

Test procedure

3.2, 2.0, 1.6, 0.8, 0.4, 0.2 and 0.1 μg/ml concentrations of ozonized olive oil were prepared in DMSO and added to spore suspensions of each fungus for 2 min. The control remained without treatment. In a parallel experiment, different concentrations of gaseous ozone (20, 16, 12, 8, 4, 2 and 0.5 μg/ml) were passed through the culture plates of each tested fungi for 2 h. The control remained without exposure. After exposure, the microconidia were harvested and adjusted to 8×10^4 conidia/ml.

Minimum inhibitory concentration (MIC)

MIC endpoints for growth were performed by plating 0.01 ml of a 1:10 dilution of each adjusted inoculum on SDA plates. The plates were incubated and then examined for the presence of fungal colonies. For MIC endpoints for spore germination, a drop of a 1:400 dilution of each adjusted inoculum was transferred to a glass slide. The MICs were determined as 80% growth and germination inhibition was compared with the control.

Effect of the MICs of ozonized oil on sporulation of fungi

Four spore suspension tubes of each tested fungi were prepared. Two of these tubes were treated for 2 min with the MIC of ozonized oil (specific for each fungus) and the other two tubes remained without treatment and were considered as control. Conidial germination was counted as log cfu/ml.

Effect of the MICs of ozonized oil on mycelium permeability

The mycelium of tested dermatophytes "previously treated with MIC (for growth) of ozonized oil" was filtered off and washed thoroughly with sterile distilled water.

Measurement of leakage of electrolytes
The method adopted by Emam was used and the result was expressed as μmohs/g fresh weight.[10]

Measurement of sugar leakage
Leakage from mycelium was determined using the anthrone sulfuric acid method described by Fales and modified by Badour. Sugar amount was expressed as μg/ml and the result was tabulated as % increase in sugar permeability.[11,12]

Effect of the MICs of ozonized oil on the activity of some enzymes secreted by tested fungi

An inoculum from each organism "treated with its specific MIC of ozonized oil or control" was inoculated into enzyme induction medium. At the end of the growth period, the fungal mycelium and the residual hair were removed and the culture filtrates were tested for enzyme assay.[13]

Keratinolytic activity was measured by the method of Yu et al., urease activity was measured using the method of Weatherburn with some modifications. Alkaline phosphatase activity was measured by the method of Harsanyit and Dorn. Amylase activity by the method of Kaufman and Tietz, lipase enzyme activity by the method of Lott et al. The results of all were tabulated as % reduction of its activity.[14–19]

Results

Minimum inhibitory concentration (MIC) for growth and spore germination

In Table 1, the MICs for fungal growth and spore germination are shown in presence of gaseous ozone and ozonized oil. The MICs were as high as 16 g/ml and 8 g/ml for both *T. interdigitale*