Effects of ozonated water on *Candida albicans* oral isolates

Ivan da Silva de Faria¹
Mariko Ueno¹
Cristiane Yumi Koga-Ito²
Wilfredo Irrazabal Urruchi³
Ivan Balducci²
Antonio Olavo Cardoso Jorge¹,²

¹Department of Odontology, Taubate University
²Department of Oral Biosciences and Diagnosis, São José dos Campos School of Dentistry, UNESP
³Department of Physics, Instituto Tecnológico da Aeronáutica, SP, Brazil

Received for publication: March 03, 2005
Accepted: August 12, 2005

Abstract

Forty-nine *C. albicans* freshly isolated strains and *C. albicans* ATCC 18804 were included in the study. Initially, suspensions containing 5 x 10⁸ viable cells/ml were prepared. This suspension was submitted to a concentration of 3.3 mg/ml of ozone for 20s, 40s, 60s, 80s, 100s, 120s, 140s, 160s, 180s and 300s. Before the experiment and also after each period of exposition, 0.1 ml of dilutions of the final suspension was plated on Sabouraud dextrose agar and incubated at 37°C for 24-48 h. After this period, the number of colony forming units was calculated. Data were statistically analyzed by Pearson’s correlation test. A reduction of the microorganism’s concentration during the contact time with ozone was observed. Complete inactivation was observed only for standard strain after 5 min. Freshly isolated *C. albicans* strains showed higher resistance to ozone. A linear correlation between the logarithm of yeast concentration and period of contact was found in both cases, being the linear correlation coefficients significant in the experiments.

Key Words:

ozone, *Candida albicans*, mouth

Correspondence to:
Antonio Olavo Cardoso Jorge
Faculdade de Odontologia de São José dos Campos/ UNESP
Av. Eng. Francisco Jose Longo, 777
São José dos Campos, SP
CEP: 12 245-000,
E-mail: cristianeykito@directnet.com
Introduction

Ozone presents several properties that can be useful in medical fields. The property of ozone to inactivate different species of microorganism has been described in the literature. In fact, the bactericide, virucide, and fungicide effects are known since the beginning of the XX century and have been employed in the treatment of infected local lesions. Ozone is a powerful oxidative agent and presents greater bactericide properties when compared to chloride and with the advantage of presenting lower toxicity. Its activity is related to the interference on bacterial growth and viral inactivation. A positive effect of ozonated sunflower seeds oil in the treatment of herpetic gingivostomatitis and alveolitis has been also related. Baysan et al. concluded that the exposition of Streptococcus mutans and S. sobrinus for a period of 10 seconds was able to reduce significantly their number in vitro. Velano et al. analysed the effect of ozonated water on Staphylococcus aureus and concluded that a period of 30 minutes of exposition was effective to inactivate this microorganism. Murakami et al. and Oizumi et al. concluded that ozone could be useful in dentures’ disinfection. Candida albicans is the most frequently fungus isolated species from human infections (80-90%) and is responsible for 75% of neonatal infections. Oral Candida spp. levels control may be an important preventive measure since the occurrence of oral candidosis may be considered a potential risk for the occurrence of systemic disease especially among immunocompromised patients. Considering that the use of antifungal therapy is related to the appearance of resistant isolates, new alternatives are necessary.

A previous study related the inactivation of C. albicans standard strain (ATCC 22572) by ozone after 5 minutes of exposition. However, studies including clinical oral isolates are not found in the literature. The aim of this study was to evaluate the effects of ozone on C. albicans strains freshly isolated from the oral cavity.

Material and Methods

This study was previously submitted and approved by the São José dos Campos School of Dentistry’s Ethic Committee (Protocol number 058/2000 – PH/CEP). Hundred-eighty healthy students at Taubaté University (Taubaté, São Paulo, Brazil), aged between 18 to 25 years old, males or females, were included in the study. The individuals who relate any systemic disease or use of antibiotics during the six months that preceded the sample’s collection were excluded.

From each individual, 2 mL of saliva were collected in a sterile universal container without stimulation. Immediately after the collection, 0.1 mL of the saliva was plated in duplicate on Sabouraud Dextrose Agar (Difco, Detroit, USA) supplemented with chloramphenicol (0.1 mg/mL, Carlo Erba, Brazil). Then, the plates were incubated for 48 h at 37°C and 5 days at room temperature.

After this period, microscopic confirmation of Candida suggestive colonies was performed. These colonies were transferred to tubes containing Sabouraud Dextrose Agar and stored at 4°C until identification procedures. Each strain was identified according to the methodology proposed by Sandvén and by Williams and Lewis. Forty-nine freshly isolated C. albicans strains were included in the study. The C. albicans type-strain ATCC 18804 was also tested for comparison purposes. The experiment with C. albicans standard strain was repeated 10 times.

Candida albicans inactivation was carried out in a 250 mL reactor by bubbling 3.3 mg/L ozone at 3L/min flowrate into the yeast suspension. The ozone generator (Ozone, mod. MVO-UV, Anceros) was developed in the Aeronautics Technologic Institute (ITA, São José dos Campos, Brazil, Department of Physics). Temperature was maintained at 25°C and the initial pH was 7.0 in all cases.

Twenty-four hours before the realization of the experiment, C. albicans isolates were plated on Sabouraud Dextrose Agar (Difco, Detroit, USA) and incubated at 37°C for 24 h. After this period, yeast cells were washed twice in PBS (pH=7.2). Standardized suspensions of each strain containing 5 x 10^4 viable cells per mL were prepared in sterile saline solution with the aid of a hemocytometer. Then, two mL of this suspension were transferred to 248 mL of sterile distilled water. This final suspension was submitted to ozonation process and an aliquot of 2 mL was collected at the following period of time: 20, 40, 60, 80, 100, 120, 140, 160, 180 and 300 seconds and transferred to tubes containing 1 mL of 0.01M sodium thiosulphate for blocking ozone’s activity. Immediately, decimal dilutions of this suspension were obtained in sterile saline solution. Then, 0.1 mL of each dilution was plated on Sabouraud Dextrose Agar and incubated for 48 h at 37°C. After the incubation period, the number of colony forming units (CFU) was counted and the logarithm of CFU/mL was calculated.

Data of log CFU/mL of C. albicans and period of exposition to ozone were statistically analyzed by Pearson’s correlation test.

Results

The curve of inactivation of C. albicans ATCC 18804 standard strain and freshly isolated strains in relation to the time of ozone exposure is presented in Figure 1. A reduction of the microorganism concentration during the period of time: 20, 40, 60, 80, 100, 120, 140, 160, 180 and 300 seconds and transferred to tubes containing 1 mL of 0.01M sodium thiosulphate for blocking ozone’s activity. Immediately, decimal dilutions of this suspension were obtained in sterile saline solution. Then, 0.1 mL of each dilution was plated on Sabouraud Dextrose Agar and incubated for 48 h at 37°C. After the incubation period, the number of colony forming units (CFU) was counted and the logarithm of CFU/mL was calculated.

Data of log CFU/mL of C. albicans and period of exposition to ozone were statistically analyzed by Pearson’s correlation test.

The curve of inactivation of C. albicans ATCC 18804 standard strain and freshly isolated strains in relation to the time of ozone exposure is presented in Figure 1. A reduction of the microorganism concentration during the contact time with ozone was observed. Complete fungicide effect of type-strain was observed only after 300 seconds. Freshly isolated C. albicans strains showed higher resistance to ozone. A linear correlation between the logarithm of yeast concentration and contact time was found in both cases. The linear correlation coefficients were significant in the experiments (C. albicans ATCC 18804: p = -0.973; freshly isolated strains: p = - 0.961).
Discussion
Ozone exposure of \textit{C. albicans} isolates demonstrated significant yeasts’ counts reduction that was significantly correlated to the time of exposition. The concentration of ozone employed to inactivate \(10^7\) yeast cells/mL was 3.3 mg/L. This concentration was higher than that effective for bacterial strains. Gurley\(^6\) observed total inactivation of suspensions with \(10^0\) and \(10^7\) \textit{Staphylococcus aureus} cells/mL with ozone concentration of 0.3 and 2.61 mg/mL, respectively. Velano et al.\(^9\) related a period of \textit{S. aureus} inactivation of 30 minutes by the exposition to 0.6 mg/mL ozonated water. Yamayoshi and Tatsumi\(^{17}\) demonstrated that ozone is a strong oxidiser for cell walls and cytoplasmatic membranes of bacteria.

Under our experimental conditions, total inactivation of \textit{C. albicans} type-strain was observed after 5 minutes of ozone exposition. These data are similar to those related by Restaino et al.\(^1\) who observed the total inactivation of \(10^4\)-\(10^6\) \textit{C. albicans} ATCC 22572 cells/mL suspension after 5 minutes of exposition to 0.33 to 2.35 mg/L. Rapid inactivation of microorganisms by ozone exposure was previously related by Baysan et al.\(^5\) and is probably due to its quickly dissipation in water.

Ozone presented fungicide activity on type-strain, however the exposition for 5 minutes was not effective for total inactivation of clinical \textit{C. albicans} isolates. These results suggest that clinical strains are more resistant to ozone in relation to the type-strain. Other experimental models may clarify if the results obtained in this study with planctonic cells may be also observed under other conditions.

In Dentistry, ozonated water has been indicated as a mouthrinse during dental surgery or after surgery procedures to promote haemostasis, to improve local oxygen supply, and to inhibit bacterial proliferation\(^{6,18-19}\). Also, it could be indicated for the decontamination of water distribution system in dental offices\(^{6,20}\). Murakami et al.\(^10\) and Oizumi et al.\(^{11}\) reported that ozone could be useful for denture’s disinfection. However, no standard protocol for these indications was previously established.

Control of \textit{C. albicans} levels is particularly desirable under systemic mycoses predisposing situations and refractory infections. The approaches based on the use of prophylactic antifungal therapy for the prevention of systemic mycoses mainly in immunocompromised patients may have contributed to the selection of resistant stains\(^{21-22}\), reinforcing the need of alternative therapy measures.

Considering the low citotoxicity of ozonated water, as reported by Nagayoshi et al.\(^{23}\), our findings may represent promising alternative measure for \textit{C. albicans} levels’ control \textit{in vivo}. However, much more research is needed to indicate ozonated water for clinical or homely uses, particularly regarding the optimal ozone concentration, mode of use and homely-use ozone generator. Also, other studies are needed to evaluate for how long time ozone maintain its effectiveness on yeasts.

References