ScienceDirect

Photodiagnosis and Photodynamic Therapy

Available online 6 May 2023, 103600

In Press, Journal Pre-proof What's this?

Effect of Antimicrobial Photodynamic Therapy, using Toluidine blue on dual-species biofilms of *Candida albicans* and *Candida krusei*

Ana Beatriz Furtado Rodrigues, Juliene Cristina da Silva Passos, Maricilia Silva Costa

Show more

Share Cite

https://doi.org/10.1016/j.pdpdt.2023.103600

Get rights and content

Abstract

Background

Although *Candida albicans* is the most frequent etiological agent of candidiasis, it has been reported a sizable number of infections related to the non-*albicans Candida* (NAC) species, *Candida krusei.* In addition, dual biofilms (biofilms composed by two species) may easily occur in vivo, becoming even more challenging the treatment of an infection. The fungicide effect of Photodynamic Therapy (PDT), using toluidine blue O (TBO) on both *C. albicans* and *C. krusei* development has been demonstrated. Thus, the objective of this study was to investigate the effects of PDT on dual-species biofilms of *Candida albicans* and *Candida krusei*.

Methods

The effect of PDT was observed on the metabolic activity of mature dual-species biofilms of *Candida albicans* and *Candida krusei* by a metabolic assay based on the reduction of XTT (2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide sodium salt) assay and the identification of *Candida albicans* and *Candida krusei* was performed on CHROMagar Candida medium.

Results

it was observed a reduction of ~30% in the metabolic activity of a mature biofilm treated with PDT, using 0.05mg·mL^{-1} TBO and during biofilm formation a predominance of *C. albicans* on *C. krusei* was observed. The inhibition observed was related to reduction in the number of Colony Forming Units (CFU) of *Candida albicans* from 31.33 ± 3.7 to 17.0 ± 1.5. The number of CFU of *C. krusei* was not significantly modified.

Conclusions

These results demonstrated the efficiency of PDT in inhibiting the dual-species biofilms of *Candida albicans* and *Candida krusei* by reducing *C. albicans* development.

Introduction

It has been demonstrated that in the world over one million deaths per year have been related to invasive fungal infections [1]. Furthermore, Candida infection is one of the most common fungal producing infections globally [2], and the opportunistic fungus *Candida albicans* is amongst the most isolated from various types of infection. This fungus is part of the human microbiota and can be found in the oral cavity mucosa, as well as the female urogenital tract [3,4]. It is a commensal microorganism easily found in healthy individuals, where it does not cause harm or disease, however if the environment allows it, whether it happens because of prolonged antibiotic therapy or other immunologic-related issues, *C. albicans* can produce an infection and be disseminated to other sites of the human body [4], [5], [6], [7].

Although *C. albicans* is the most frequent etiological agent of candidiasis, it has been reported a significant number of infections related to the non-*albicans Candida* (NAC) specie, *Candida krusei* [8], [9], [10].

Candida krusei is recognized as one of the most resistant species within the genus because of its intrinsic resistance to antifungal drugs currently available, such as fluconazole [7,11]. In addition, a high mortality rate (40–58%) was observed in infections produced by *C. krusei* [12]. This fungus can be found as a transitory microorganism in different sites of the human microbiota, but it is commonly isolated from the oral mucosa [7,13]. Candidiasis produced by this species are emergent, given the severity of the causes and the difficulties in the treatment. It is usually observed in cylindrical shape, and its colony morphology white and round is similar to other yeasts [7,12].

Both microorganisms have been largely studied for years, mainly because of their ability to cause severe infections, but also because of the ability to form biofilms on biotic and/or abiotic surfaces [3,7,12]. Biofilms are communities of microorganisms produced as a protection mechanism, but it also works as a virulence factor because of their ability to escape from the immunologic system and the fact that conventional antifungal drugs are not able to pass through the superficial layers and eradicate the cells [5,7,11,12,14].

They have been for the past years one of the most important challenges in the healthcare systems. Biofilms are complex aggregates that are formed initially by planktonic cells. These cells, free in the environment, adhere to a surface, whether human or animal tissue, and from then on they begin to produce substances that allow the biofilm to be formed, such as microcolonies, hyphae or pseudo hyphae [5,12,14,15]. The adhesion phase is the only one reversible. Once the biofilm is adhered, it becomes extremely hard to remove it. After the adhesion and the microcolony formation, the next phase consists of the biofilm maturation. In this phase occurs the extracellular matrix (ECM) formation [3,14,15]. This is a crucial step because the ECM protects the microorganism against antimicrobial drugs and the cells of the host's immune system [14,15].

In addition, biofilms can be formed by one single species or more [15,16]. Dual (composed by two species) or polymicrobial (composed by three or more species) biofilms may easily occur in vivo because the human body is colonized by hundreds of different microorganisms. In these cases, where two or more species coexist in a unique environment, it becomes even more challenging the treatment of an infection because of the need to apply higher doses of a drug increases along with the risks for the patient. It is known that antifungal drugs, especially if used for extended periods can harm kidney and liver cells given their toxicity [3,16,17]. Therefore, it is extremely necessary to develop new ways to treat infections caused by fungi, especially the ones accompanied by biofilms.

Photodynamic Therapy (PDT) has been used as a safe alternative to inactivate microorganisms and cancerous cells for years given its ability to cause irreversible damage to target cells [11,18,19]. This modality of therapy is based on the association of visible light, a non-toxic photosensitizer (PS) and molecular oxygen [17], [18], [19], producing reactive oxygen species (ROS). These products are highly reactive to biological

components, damaging several vital components of the microorganism, such as DNA, proteins, lipids, resulting in cell death [13,[17], [18], [19], [20], [21], [22]].

It has been demonstrated the fungicide effect of PDT, using toluidine blue-O (TBO), a phenothiazinium salt, on *C. albicans* [4,5,19,20,23,24]. Moreover, the effect of PDT using TBO on *C. krusei* was more recently demonstrated [4,11,19]. Taken together, these results demonstrate the ability of PDT using TBO to inhibit both *C. albicans* and *C. krusei* development, leading us to consider the potential of PDT in modifying the development of biofilms formed by two species of Candida. Thus, the objective of this study was to investigate the effects of PDT, using TBO, on mature dual-species biofilms of *Candida albicans* and *Candida krusei*.

Section snippets

Organisms and growth conditions

Cultures of both *Candida albicans* strain ATCC 10231 and *Candida krusei* strain ATCC 6258 were plated on Sabouraud dextrose agar (Merck, Darmstadt, Hesse, Germany) and incubated in atmospheric air, at 37°C. After 48 hours of incubation, a sample of the colonies was removed from the surface of the agar plate and suspended in sterile physiological solution (0.85 % NaCl), at a cell density of 10^7 cell·mL^{-1}, determined using the Neubauer chamber in the presence of the vital dye, toluidine blue-O (0.5 …

Results

Initially, the effect of PDT was observed on the metabolic activity of dual-species biofilms of *Candida albicans* and *Candida krusei*. It was observed an inhibition of ~30 % in the metabolic activity of mature biofilms treated with PDT, using 0.05 mg·mL^{-1} TB (Fig. 1). PDT using TBO in concentrations varying from 0.02 to 0.1 mg·mL^{-1} produced a reduction of the metabolic activity of the biofilm between 22 and 33%. Biofilms either only irradiated or in the presence of TBO, but not irradiated, were…

Discussion

It has been observed in the recent decade that an alarming number of microorganisms have been presenting resistance to the conventional drug therapies employed. Thus, it is necessary to investigate new alternatives that may contribute to the inactivation of microorganisms [47]. In this scenario, PDT emerged as a safe and accessible alternative to help in the process of inactivating microorganisms that are difficult to treat with the methods currently at reach.

Fungi infections are known to be…

Role of funding source

The authors would like to thank FAPESP for the financial support.…

Ethical Approval

In this study, all experiments were performed using Cultures of *Candida albicans* (ATCC 10231) and *Candida krusei* strain ATCC 6258, therefore it is not necessary to have approval by local authorities.…

Informed Consent

We have obtained permission from all the authors, we declare that the material has not been published in whole or in part elsewhere, the paper is not currently being considered for publication elsewhere.…

CRediT authorship contribution statement

Ana Beatriz Furtado Rodrigues: Writing – original draft, Resources, Methodology, Investigation. **Juliene Cristina da Silva Passos:** . **Maricilia Silva Costa:** ….

Declaration of Competing Interest

The authors have no financial, personal, or other conflicts of interest related to this work.…

Acknowledgments

This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.…

References (58)

J.C. Carmello *et al.*
Photoinactivation of single and mixed biofilms of Candida albicans and non-albicans Candida species using Phorodithazine®
Photodiagnosis Photodyn Ther (2017)

R. Rajendran
Biofilm formation is a risk factor for mortality in patients with Candida albicans bloodstream infection—Scotland, 2012–2013
Clinical Microbiology and Infection (2016)

M. Pourhajibagher *et al.*
Sub-lethal doses of photodynamic therapy affect biofilm formation ability and metabolic activity of Enterococcus faecalis
Photodiagnosis Photodyn Ther (2016)

D. Wohlmeister
Differentiation of Candida albicans, Candida glabrata, and Candida krusei by FT-IR and chemometrics by CHROMagar™ Candida
J Microbiol Methods (2017)

I.B. Rosseti *et al.*
Diphenyl diselenide (PhSe)2 inhibits biofilm formation by Candida albicans, increasing both ROS production and membrane permeability
Journal of Trace Elements in Medicine and Biology (2015)

Y. Li
Hydrogen peroxide potentiates antimicrobial photodynamic therapy in eliminating Candida albicans and Streptococcus mutans dual-species biofilm from denture base

Photodiagnosis Photodyn Ther (2022)

K. Honraet *et al.*

Comparison of three assays for the quantification of Candida biomass in suspension and CDC reactor grown biofilms

J Microbiol Methods (2005)

T. Gobor

Proposal of protocols using D-glutamine to optimize the 2,3-bis(2-methoxy-4-nitro-5-sulfophenly)-5-[(phenylamino) carbonyl]-2H-tetrazolium hydroxide (XTT) assay for indirect estimation of microbial loads in biofilms of medical importance

J Microbiol Methods (2011)

E. Peeters *et al.*

Comparison of multiple methods for quantification of microbial biofilms grown in microtiter plates

J Microbiol Methods (2008)

L. de Carvalho Leonel *et al.*

Photodynamic Antimicrobial Chemotherapy (PACT) using methylene blue inhibits the viability of the biofilm produced by Candida albicans

Photodiagnosis Photodyn Ther (2019)

View more references

Cited by (0)

Recommended articles (0)

View full text