Photodiagnosis and Photodynamic Therapy 18 (2017) 232–235

Contents lists available at ScienceDirect

Photodiagnosis and Photodynamic Therapy

journal homepage: www.elsevier.com/locate/pdpdt

Selective photoinactivation of *Histoplasma capsulatum* by water-soluble derivatives chalcones

Wanessa C.M.A. Melo[a], Mariana Bastos dos Santos[b], Beatriz de Carvalho Marques[b], Luis Octávio Regasini[b], Maria José Soares Mendes Giannini[a], Ana Marisa Fusco Almeida[a,*]

[a] *Biociências e Biotecnologia Aplicada a Farmácia/Núcleo Proteomica, Universidade Estadual Paulista, Araraquara-SP, Brazil*
[b] *Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista, São José do Rio-SP, Brazil*

ARTICLE INFO

Article history:
Received 13 October 2016
Received in revised form 24 January 2017
Accepted 9 March 2017
Available online 27 March 2017

Keywords:
Chalcone
Histoplasma capsulatum
aPDT
Selectivity

ABSTRACT

Histoplasmosis is a respiratory and systemic disease caused by the dimorphic fungus *Histoplasma capsulatum*. The clinical features may vary from asymptomatic infections to disseminated severe form depending of patient immunity. The treatment of histoplasmosis can be performed with itraconazole, fluconazole, and in the disseminated forms is used amphotericin B. However, the critical side effects of amphotericin B, the cases of itraconazole therapy failure and the appearance of fluconozole-resistant strains makes necessary the search of new strategies to treat this disease. Antimicrobial photodynamic therapy (aPDT) seems to be a potential candidate once have been show efficacy to inhibit others dimorphic fungi. Although the photosensitizer (PS) chalcone aggregates in biological medium, it has antifungal activity and show a high quantum yield of ROS formation. So, the aim of this study was to obtain the experimental parameters to achieve an acceptable selective chalcone water-soluble derivatives photoinactivation of *H. capsulatum* comparing with fibroblastic and keratinocytes cells which are the constituents of some potential host tissues. Yeast and cells were incubated with the same chalchones concentrations and short incubation time followed by irradiation with equal dose of light. The best conditions to kill *H. capsulatum* selectively were very low photosensitizers concentration (1.95 $\mu g\,mL^{-1}$) incubated by 15 min and irradiated with LED 450 nm with 24 $J\,cm^{-2}$. Key words: chalcone, Histoplasma capsulatum, aPDT, selectivity.

1. Background

Histoplasmosis is a fungal infection caused by the dimorphic fungus *Histoplasma capsulatum* [1]. Depending on host immunity the disease can effect organs phagocytic mononuclear system such as spleen and liver [2,3], as well as can create oral and tissue lesions [4]. The treatment of histoplasmosis can be performed with itraconazole, fluconazole and in the disseminated form amphotericin B [5]. However, the appearance of fluconazole-resistant strains [6,7], cases of failure with itraconazole therapy [8] and that amphotericin B causes severe side effects, including nephrotoxicity and hepatotoxicity [9] makes urgent the development of new strategies to treat this disease. Antimicrobial photodynamic therapy (aPDT) seems to be a potential candidate since it has been shown to effectively kill different species of fungi, causing minimal damage to host cells [10,11].

2. Aims

Several photosensitizers (PSs) have been investigated for aPDT applications in the treatment and control of yeasts, including chalcone that show a high quantum yield of reactive species of oxygen (ROS) formation [12]. Nevertheless, the chalcones in water and biological media forms aggregates or precipitates that prevent its efficient photoactivity [13,14]. So, in this study we inedited investigate the selective photoinactivation of *H. capsulatum* using chalcone water-soluble derivatives.

3. Materials and Methods

3.1. Histoplasma capsulatum *Strain and Culture Conditions*

H. capsulatum (ATCC #26029) was grown aerobically in brain-heart infusion (BHI-broth) (DifcoTM) at 37 °C and 80 rpm, until

* Corresponding author.
E-mail addresses: almeidaf@fcfar.unesp.br, ana.marisa@uol.com.br (A.M.F. Almeida).

http://dx.doi.org/10.1016/j.pdpdt.2017.03.001
1572-1000/

logarithm phase. The yeast was harvested by centrifugation (5 min, 4000 rpm) and resuspended in sterile phosphate buffered saline (PBS) at pH 7.0 to final concentration of 1×10^7 cell mL^{-1}.

3.2. Mammalian Cell Culture

NOK – human oral keratinocyte cell line, HaCat – a keratinocyte cell line from adult human skin and MRC-5 a human lung fibroblast were used as model cells. The cell lines were cultured in Dulbecco's modified minimal essential medium (D-MEM, Sigma) containing 10% fetal bovine serum (Cultilab), penicillin 10,000 U.I. mL^{-1}, streptomycin 10 mg mL^{-1} and 25 μg mL^{-1} amphotericin B (Sigma) as monolayers adhered to the plastic bottles at 37 °C, 5% CO_2 and 95% air atmosphere.

3.3. Photosensitizer and Light Source

Chalcones used in the present investigation were prepared by chemical synthesis in the laboratories of Instituto de Biociências, Letras e Ciências Exatas, Universidade Estadual Paulista (São José do Rio-SP, Brazil), solubilized in dimethyl sulfoxide (DMSO) at a concentration of 1 mg mL^{-1}, aliquoted and stored at −20 °C.

The light source used was an illumination diffusion table composed by blue LEDs (predominantly 450 nm), named Bio table and gently assigned by Dra. Carla Raquel Fontana Mendonça, Universidade Estadual Paulista, Araraquara-SP, Brazil). The output power was maintained constant at 34 mW cm^{-2} and the fluence of 12 and 24 J cm^{-2} was obtained by ~6 and 12 min of illumination, respectively.

3.4. aPDT Procedure

Suspensions of 100 μL of *H. capsulatum* (10^7 cell mL^{-1}) were transferred into wells of a microtiter plate. Identical volume of chalcone water-soluble derivatives in different concentrations (0–62.5 μg mL^{-1}) was added to each well. After the incubation time of 15 min at 37 °C in the dark, the samples were irradiated at 450 nm with a light dose of 12 and 24 J cm^{-2}. Cell viability was determined by using the micro drop technique and the colony counting were performed after 48 h of incubation at 37 °C in order to calculate the survival index (SI).

The same conditions were applied to mammalian cells, however, the cells viability was performed using MTT-microculture tetrazolium assay, a method of assessing cellular response to aPDT [15,16].

3.5. Statistics

Values are given as means and standard errors of four separate experiments. Differences between killing curves were tested for significance by ANOVA.

4. Results and Discussion

The aPDT efficacy is dependent of the PS amount inside the microorganism or binding with the microorganism, *i.e.*, the pharmacodynamic of the dye [17]. Due that the chalcones used in this study were functionalized with hydroxyl group in order to increase their solubility in water or biologically compatible media and easily binding with the microorganisms. This functionalization also imparts a higher ability to produce singlet oxygen, hydroxyl radicals and superoxide anion upon illumination [18]. Fig. 1 presents the absorption bands of the chalcones derivatives (named CH1 and CH2) as well as its chemical structure. The extinction coefficients of the chalcones derivatives were CH2 > CH1. In addition, the

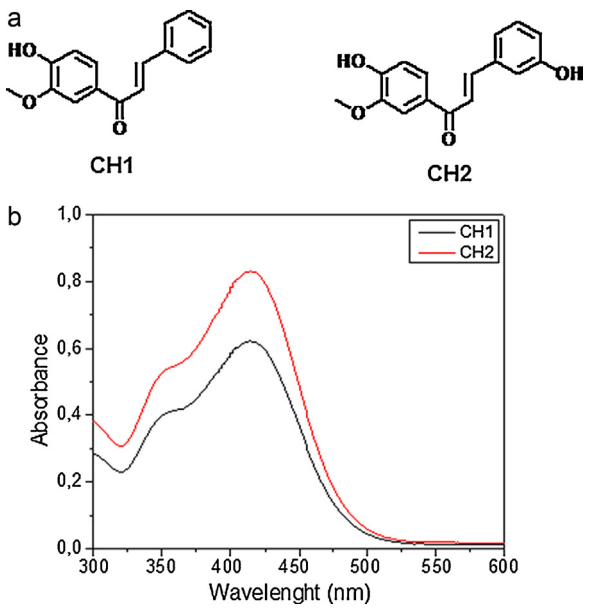

Fig. 1. Chemical structures (a) and absorption spectra (b) of CH1 and CH2 solution 1.0 μg mL^{-1} in DMSO.

structural modification of the chalcone did not promote the photochemical properties of the PS which maintained its maximum fluorescence emission spectrum at 450 nm.

Although the fungi present a complex structure it is known that the response of such cells to photodynamic processes is less strictly controlled by structural factors as compared with bacteria [11,19]. However, the similarities with mammalian cells should be considered, mainly due the PS functionalization increase the binding with the host cells and may unfeasible the selectivity photoinactivation. So, to identify those structural features we evaluated the effect of the chalcones water-soluble derivatives to promote a selective photoinactivation of the dimorphic fungus *H. capsulatum* (Figs. 2 and 3).

According to the methodology described by Soncin et al. [20], we performed preliminary studies to evaluated the binding of chalcones with microbial in comparison with mammalian cells. The results of this study (Supplementary Table 1) were used to define the time incubation of aPDT experiments. Independent of PSs concentration, it was observed a continuous and fast accumulation of chalcones into the microbial and mammalian cells up to first 5 min. However, after 15 min of incubation the amount of fungi-associated PSs reached a plateau value, while the mammalian cells prolonging the PSs accumulation for more than 60 min. In addition, the recovered concentration of PSs into mammalian cells was three times less than into *H. capsulatum*, after 15 min of incubation. So, within this results we may suggest that was possible obtain a selective PSs accumulation.

The accumulation of CH2 into all cells was higher than CH1, probably due the difference of chemical structure between them which makes the CH2 more hydrophilic and consequently decreases the aggregation. This fact also contributed to increase the CH2 photodynamic activity compared to CH1, once the complete *H. capsulatum* inhibition was reached for CH2 using less PS concentration and light dose than CH1 (Figs. 2 and 3). The CH2 at 7.81 μg mL^{-1} associated to 24 J cm^{-2} light dose was capable to reduce 100% (seven Log reduction) of *H. capsulatum* while was necessary eight times of CH1 concentration to inhibit 95% (6.95 Log reduction) of this fungus at same aPDT conditions. Suggesting that the PS hidrophilicity besides allows the intracellular accumulation may promote the increase of ROS production.

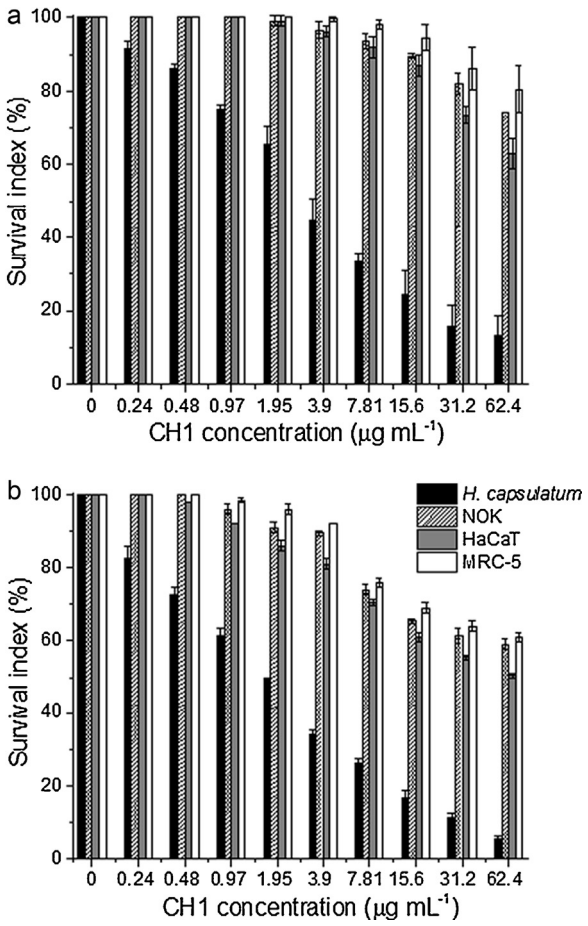

Fig. 2. Survival index of *H. capsulatum*, NOK, HaCaT and MRC-5 after 15 min of incubation with different concentrations of chalcone water-soluble derivative (CH1) upon irradiation at 450 nm (a) 12 J cm^{-2} and (b) 24 J cm^{-2}).

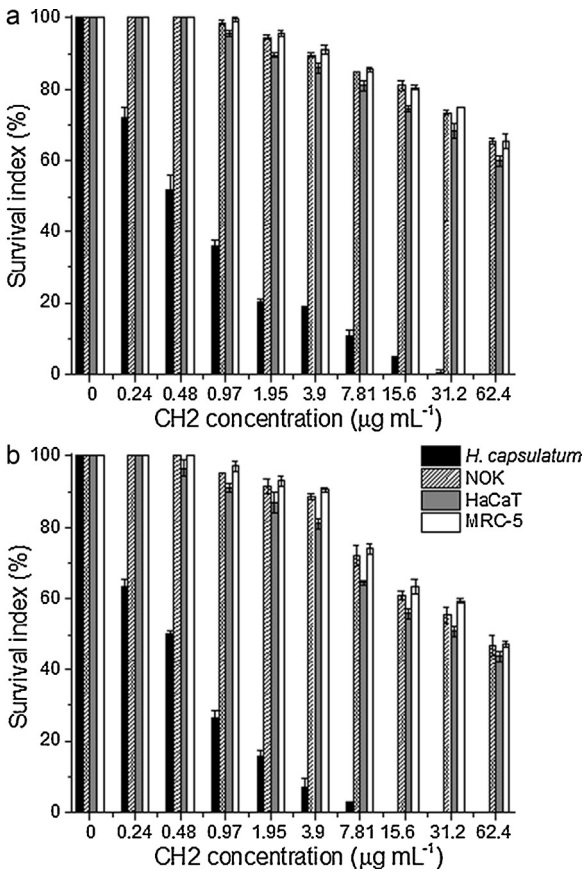

Fig. 3. Survival index of *H. capsulatum*, NOK, HaCaT and MRC-5 after 15 min of incubation with different concentrations of chalcone water-soluble derivative (CH2) upon irradiation at 450 nm (a) 12 J cm^{-2} and (b) 24 J cm^{-2}).

In general, it was observed that only the blue light or the PSs (0.24–15.65 μg mL^{-1}) in the dark was not capable to cause any inhibition for both fungus and host cells. In the other hand, the association of light – chalcones promoted a significant inhibition of *H. capsulatum* which was proportional to the increase of PSs concentration and light dose, especially by CH2.

The aPDT in low concentrations of the PSs (0.24–3.9 μg mL^{-1}) did not affect significantly the mammalian cells, especially comparing the effect of aPDT against fungus cell. If we compare the effect of aPDT between the mammalian cells, is possible observed that the keratinocyte cells were more susceptible than fibroblast cell, probably due MRC-5 exhibit a morphologic structure more complex once presents extracellular matrix that increases the protection against ROS [21].

So, the presented results showed that the chalcones are capable to inhibit *H. capsulatum* with minimal damage to host cells. The best aPDT condition to achieve this selectivity was using the CH2 as photosensitizer at 1.95 μg mL^{-1}, 15 min of time incubation and 24 J cm^{-2} of light dose, promoting ~85% (5.85 Log reduction) of *H. capsulatum* reduction while the fibroblast and keratinocytes cells showed 93 and 90% of SI, respectively. We also may highlighted that the CH2 at higher concentration (31.2 and 62.4 μg mL^{-1}) and a low light dose (12 J cm^{-2}) was capable to inhibit 100% of the fungus and the reduction in SI of mammalian cells was up to 40%. This damage to host cells is much less than caused by amphotericin B at lowest concentration (5 μg mL^{-1}), which induce a complete reduction of mammalian cells SI [22]. So, the chalcone water-soluble derivatives have great potential to be used in aPDT against *H. capsulatum*.

5. Conclusions

Several authors have been reported the *in vitro* action of aPDT on yeasts with several photosensitizers. As far as we know, no data are available on the efficacy of this therapy against the *H. capsulatum*. Within the results obtained we observed that low concentration of chalcone water-soluble derivatives can promote a total inhibition of this fungus using only 15 min of incubation and low light doses (12 and 24 J cm^{-2}). Furthermore, this study suggests that the hidrophilicity of chalcones may be the main responsible for that great inhibition, once this characteristic promoted an increase of PS accumulation into microbial cell.

The damage caused against the host cells was not significant, which allowed to achieve the selective photoinactivation of this fungus. So, this can be a first step to get a protocol to treat histoplasmosis using aPDT, especially in the secondary infection (oral mucosa and skin).

Acknowledgements

The authors expresses sincere thanks for the financial support from CAPES, FAPESP, Cnpq and Programa de Apoio ao Desenvolvimento Científico da Faculdade de Ciências Farmacêuticas da UNESP (PADC).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.pdpdt.2017.03.001.

References

[1] N.S. Pitangui, J.C. Sardi, J.F. Silva, T. Benaducci, R.A. Moraes da Silva, G. Rodriguez-Arellanes, M.L. Taylor, M.J. Mendes-Giannini, A.M. Fusco-Almeida, Adhesion of *Histoplasma capsulatum* to pneumocytes and biofilm formation on an abiotic surface, Biofouling 28 (7) (2012) 711–718.

[2] L. Ajello, Italian contributions to the history of general and medical mycology, Med. Mycol. 36 (Suppl 1) (1998) 1–11.

[3] M.A. Dias, R.M. Oliveira, M.C. Giudice, H.M. Netto, L.R. Jordao, I.M. Grigorio, A.R. Rosa, J. Amorim, J.D. Nosanchuk, L.R. Travassos, C.P. Taborda, Isolation of *Histoplasma capsulatum* from bats in the urban area of Sao Paulo State, Brazil, Epidemiol. Infect. 139 (10) (2011) 1642–1644.

[4] S. Vidyanath, P. Shameena, S. Sudha, R.G. Nair, Disseminated histoplasmosis with oral and cutaneous manifestations, J. Oral. Maxillofac. Pathol. 17 (1) (2013) 139–142.

[5] R.S. Brilhante, R.A. de Lima, F.J. Marques, N.F. Silva, E.P. Caetano, S. Castelo-Branco Dde, J. Bandeira Tde, J.L. Moreira, A. Cordeiro Rde, A.J. Monteiro, Z. Pires de Camargo, J.J. Sidrim, M.F. Rocha, *Histoplasma capsulatum* in planktonic and biofilm forms: in vitro susceptibility to amphotericin B, itraconazole and farnesol, J. Med. Microbiol. 64 (Pt 4) (2015) 394–399.

[6] J. Wheat, P. Marichal, H. Vanden Bossche, A. Le Monte, P. Connolly, Hypothesis on the mechanism of resistance to fluconazole in *Histoplasma capsulatum*, Antimicrob. Agents Chemother. 41 (2) (1997) 410–414.

[7] H. Vanden Bossche, F. Dromer, I. Improvisi, M. Lozano-Chiu, J.H. Rex, D. Sanglard, Antifungal drug resistance in pathogenic fungi, Med. Mycol. 1 (1998) 119–128.

[8] A.M. LeMonte, K.E. Washum, M.L. Smedema, C. Schnizlein-Bick, S.M. Kohler, L.J. Wheat, Amphotericin B combined with itraconazole or fluconazole for treatment of histoplasmosis, J. Infect. Dis. 182 (2) (2000) 545–550.

[9] R.J. Hamill, Amphotericin B formulations: a comparative review of efficacy and toxicity, Drugs 73 (9) (2013) 919–934.

[10] P. Calzavara-Pinton, M.T. Rossi, R. Sala, M. Venturini, Photodynamic antifungal chemotherapy, Photochem. Photobiol. 88 (3) (2012) 512–522.

[11] R.F. Donnelly, P.A. McCarron, M.M. Tunney, Antifungal photodynamic therapy, Microbiol. Res. 163 (1) (2008) 1–12.

[12] P. Boeck, P.C. Leal, R.A. Yunes, V.C. Filho, S. Lopez, M. Sortino, A. Escalante, R.L. Furlan, S. Zacchino, Antifungal activity and studies on mode of action of novel xanthoxyline-derived chalcones, Arch. Pharm. (Weinheim) 338 (2–3) (2005) 87–95.

[13] A. Boumendjel, J. Boccard, P.A. Carrupt, E. Nicolle, M. Blanc, A. Geze, L. Choisnard, D. Wouessidjewe, E.L. Matera, C. Dumontet, Antimitotic and antiproliferative activities of chalcones: forward structure-activity relationship, J. Med. Chem. 51 (7) (2008) 2307–2310.

[14] W.C. de Melo, P. Avci, M.N. de Oliveira, A. Gupta, D. Vecchio, M. Sadasivam, R. Chandran, Y.Y. Huang, R. Yin, L.R. Perussi, G.P. Tegos, J.R. Perussi, T. Dai, M.R. Hamblin, Photodynamic inactivation of biofilm: taking a lightly colored approach to stubborn infection, Expert Rev. Anti Infect. Ther. 11 (7) (2013) 669–693.

[15] J.L. Merlin, S. Azzi, D. Lignon, C. Ramacci, N. Zeghari, F. Guillemin, MTT assays allow quick and reliable measurement of the response of human tumour cells to photodynamic therapy, Eur. J. Cancer 28A (8–9) (1992) 1452–1458.

[16] G.P. Tegos, T.N. Demidova, D. Arcila-Lopez, H. Lee, T. Wharton, H. Gali, M.R. Hamblin, Cationic fullerenes are effective and selective antimicrobial photosensitizers, Chem. Biol. 12 (10) (2005) 1127–1135.

[17] L. Huang, M. Wang, T. Dai, F.F. Sperandio, Y.Y. Huang, Y. Xuan, L.Y. Chiang, M.R. Hamblin, Antimicrobial photodynamic therapy with decacationic monoadducts and bisadducts of [70]fullerene: in vitro and in vivo studies, Nanomedicine (Lond) 9 (2) (2014) 253–266.

[18] M.J. Garland, C.M. Cassidy, D. Woolfson, R.F. Donnelly, Designing photosensitizers for photodynamic therapy: strategies, challenges and promising developments, Future Med. Chem. 1 (4) (2009) 667–691.

[19] T. Dai, B.B. Fuchs, J.J. Coleman, R.A. Prates, C. Astrakas, T.G. St Denis, M.S. Ribeiro, E. Mylonakis, M.R. Hamblin, G.P. Tegos, Concepts and principles of photodynamic therapy as an alternative antifungal discovery platform, Front. Microbiol. 3 (2012) 120.

[20] M. Soncin, C. Fabris, A. Busetti, D. Dei, D. Nistri, G. Roncucci, G. Jori, Approaches to selectivity in the Zn(II)-phthalocyanine-photosensitized inactivation of wild-type and antibiotic-resistant Staphylococcus aureus, Photochem. Photobiol. Sci. 1 (10) (2002) 815–819.

[21] G. Bottai, R. Mancina, M. Muratori, P. Di Gennaro, T. Lotti, 17beta-estradiol protects human skin fibroblasts and keratinocytes against oxidative damage, J. Eur. Acad. Dermatol. Venereol. 27 (10) (2013) 1236–1243.

[22] S. Harmsen, A.C. McLaren, C. Pauken, R. McLemore, Amphotericin B is cytotoxic at locally delivered concentrations, Clin. Orthop. Relat. Res. 469 (11) (2011) 3016–3021.