UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE BAURU

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Evaluation of the cytotoxic effect of antimicrobial photodynamic therapy on fibroblasts (NIH/3T3) and expression of Bcl-2 family genes

Efeito citotóxico da terapia fotodinâmica antimicrobiana sobre fibroblastos (NIH/3T3) e a expressão de genes da família Bcl-2

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Orientador: Prof. Dr. Thiago Cruvinel da Silva

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FOLHA DE APROVAÇÃO

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RESUMO

A terapia fotodinâmica antimicrobiana (aPDT) tem sido utilizada como um tratamento coadjuvante de infecções bucais em abordagens de mínima intervenção clínica. Sua efetividade antimicrobiana foi demonstrada em diversos estudos. Entretanto, há uma falta de evidência sobre seu efeito citotóxico sobre células eucarióticas. O objetivo deste estudo foi avaliar o potencial citotóxico da terapia fotodinâmica antimicrobiana mediada por dois agentes fotossensibilizantes, azul de metileno e curcumina, sobre fibroblastos de camundongos. Células foram tratadas com 0,1 ou 1,0 $mg.mL^{-1}$ de azul de metileno associado ou não a um LED 630 nm, ou 0.6 ou 6 μM de curcumina associada ou não a um LED 455 nm, com densidades de energia de 0.075 ou 7.5 $J.cm^{-2}$. A viabilidade cellular foi determinada pelos ensaios de Brometo de 3-(4,5-dimetil-2-tiazolil)-2,5-difenil-2H-tetrazólio (MTT) e cristal violeta (CV). A expressão de cDNA para os genes ligados à apoptose Bax, Bad, Bcl-2, VDAC-1, citocromo C e Fas-L foi assessada por PCR quantitative (qPCR), após 1, 3, 6 e 24 h dos tratamentos. As diferenças entre os grupos foram detectadas pelos testes de Kruskal-Wallis e post-hoc de Dunn para os ensaios de MTT e CV, e pelos testes de ANOVA e post-hoc de Tukey para qPCR ($P<0.05$). A combinação de azul de metileno a 1,0 $mg.mL^{-1}$ e LED a 7.5 $J.cm^{-2}$ reduziu significativamente a viabilidade celular, o mesmo sendo observado pelas combinações de curcumina a 6 μM com LED a 0,075 e 7,5 $J.cm^{-2}$, que reduziram a viabilidade celular em 47% e 99%, respectivamente. Também, a associação de curcumina a 0,6 μM com LED a 7,5 $J.cm^{-2}$ reduziu a viabilidade de fibroblastos em 34%. A aPDT mediada por azul de metileno aumentou a expressão de citocromo C e FAS-L (3 h), e Bax/Bcl-2, Bad/Bcl-2, e VDAC-1 (6 h). aPDT mediada por curcumina aumentou significativamente a expressão relativa de Bax/Bcl-2 e dos genes citocromo C, VDAC-1, e Fas-L. Portanto, aPDT mediada por azul de metileno e curcumina induziu citotoxicidade em fibroblastos de camundongo, com consequente ativação da via de sinalização apoptótica Bcl-2. Novos estudos são necessários para determinar parâmetros adequados de aPDT para inativar microrganismos com danos mínimos às células eucarióticas hospedeiras.

Palavras-chave: Terapia Fotodinâmica, Viabilidade Celular, Apoptose

ABSTRACT

Evaluation of the cytotoxic effect of photodynamic therapy on fibroblasts (NIH/3T3) and expression of Bcl-2 family genes

Antimicrobial photodynamic therapy (aPDT) has been used as an adjuvant treatment of oral infections in a minimal intervention clinical approach. Its antimicrobial efficacy was demonstrated in several studies; however, there is a lack of evidence on its cytotoxic effects on eukaryotic cells. The aim of this study was to evaluate the cytotoxicity of aPDT mediated by two photosensitizing agents, methylene blue and curcumin, on mouse fibroblasts. Cells were treated with 0.1 or 1.0 $mg.mL^{-1}$ methylene blue (MB) associated or not to LED at 630 nm, or 0.6 or 6 μM curcumin combined or not with LED at 455 nm, with densities of 0.075 or 7.5 $J.cm^{-2}$. Cellular viability was examined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and crystal violet (CV) assays. The expression of cDNA for Bax, Bad, Bcl-2, VDAC-1, cytochrome C and Fas-L genes related to apoptosis was assessed by quantitative PCR (qPCR) after 1, 3, 6 and 24 h from treatments. The differences between groups were detected by Kruskal-Wallis and post-hoc Dunn's tests for MTT and CV assays, and by ANOVA and post-hoc Tukey test for qPCR ($P<0.05$). The combination of 1.0 $mg.mL^{-1}$ MB and 7.5 $J.cm^{-2}$ LED significantly reduced the cellular viability. The same was observed for the combinations of 6 μM curcumin plus 0.075 and 7.5 $J.cm^{-2}$ LED, which reduced viable cells in 47% and 99%, respectively. Also, the combination of 0.6 μM curcumin plus 7.5 $J.cm^{-2}$ LED reduced the viability of fibroblasts in 34%. MB-mediated aPDT increased the expression of cytochrome C and Fas-L after 3 h, and Bax/Bcl-2, Bad/Bcl-2, and VDAC-1 after 6 h from treatments. Curcumin-mediated aPDT increased significantly the relative expression of Bax/Bcl-2, cytochrome C, VDAC-1, and Fas-L genes. Therefore, MB- and curcumin-mediated aPDT induced cytotoxicity on mouse fibroblasts, with consequent activation of Bcl-2 apoptosis signaling pathways. Further studies are needed to determine the adequate parameters of aPDT to inactivate microorganisms without damaging eukaryotic cells.

Keywords: Photodynamic Therapy, Cell Viability, Apoptosis

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1 INTRODUCTION

1 **INTRODUCTION**

Initially, Photodynamic Therapy (PDT) has been focused on the development of effective protocols for cancer management (ALLISON et al., 2005, 2006). Recently, with growing antibiotic resistance, PDT emerges as antimicrobial treatments, particulary for superficial infections. Antimicrobial photodynamic therapy (aPDT) can be used as a complementary treatment for selective caries removal (GURSOY et al., 2013), periodontitis (ROVALDI et al., 2000; CHRISTOODOULIDES et al., 2008; NASTRI et al., 2010), and endodontic root disinfection (WILLIAMS et al., 2006; SOUKOS et al., 2006; BONSOR et al., 2006; FOSCHI et al., 2007). aPDT is based on the interaction between a photosensitizing agent (PS) absorbed by microorganisms cells, activated by a complementary light source that increases the molecular energy of PS and changes it to a triplet excited molecule (KONOPKA; GOSLINSKI, 2007). This molecule can form free radicals (photochemical reaction type I) or react with oxygen molecules, producing reactive oxygen species (ROS), such as singlet oxygen (photochemical reaction type II) (ABRAHAMSE; HAMBLIN, 2016). These reactions promote an oxidative stress, causing damages on cellular membrane, mitochondria, lysosomes or DNA (MAISCH,2007; TARASZKIEWICZ et al., 2013; VATANSEVER et al., 2013).

Methylene blue (MB) [chloride 3.7 bis(dimethylamino) phenothiazine-5-io] is a dye from the phenothiazinium class of compounds (TUITE, 1993), with favorable properties for aPDT, such as low molecular weight, classical $1O_2$ generator, hydrophilicity, cationic form at physiological pH and strong light absorption at 660 nm (OLIVEIRA et al., 2011; BACELLAR et al., 2015; ROSA et al., 2015). Briefly, molecules of MB incorporated to cellular structures of microorganisms are able to absorb photons and excite electrons. These electrons are transferred to a substrate or molecular oxygen, resulting in superoxides that can damage or kill microbial cells (CHIAVIELLO et al., 2011; GHARESI et al., 2017).

Curcumin (1,7-bis(4-hydroxy 3-methoxy phenyl)-1,6-heptadiene-3, 5-dione), a natural dye isolated from the rhizomes of *Curcuma longa*, have been purposed as a potential photosensitizing (PS) in aPDT. Traditionally, it is used for the treatment of cold, skin diseases, and inflammation (CHATTOPADHYAY et al., 2004;

AGGARWAL et al., 2007). Also, recent researches confirmed its potential as antitumor, antioxidant, antimicrobial and anti-inflammatory (AGGARWAL et al., 2003; CRIVELLO et al., 2005; DUVOIX et al., 2005). It has a maximum absorption with blue LED on wavelength of 430 nm, presenting a strong phototoxic effect in microorganisms even when used in lower concentrations (GHARESI et al., 2017; SAITAWEE et al., 2018). In tumor cells, the cytotoxicity of curcumin is due to the induction of apoptosis mediated by the direct release of cytochrome C, and the subsequent activation of caspases (STEINER-OLIVEIRA et al., 2015). In microorganism cells, the cytotoxicity occurs by the production of reactive oxygen species, such as singlet oxygen ($^1O_2$), hydrogen peroxide and superoxide (DAHL et al., 1989; CHIGNELL et al., 1994; DOVIGO et al., 2011).

Although the antimicrobial efficacy of aPDT was demonstrated in several studies, there is a lack of evidence on its cytotoxic effect on eukaryotic cells. Therefore, the purpose of this *in vitro* study was to evaluate the cytotoxicity and apoptotic effects of methylene-blue- and curcumin-mediated antimicrobial photodynamic therapy in different dye concentrations and light densities on mouse fibroblasts.

2 ARTICLES

2 ARTICLES

This dissertation is presented in a format of two manuscripts, written according to the instructions and guidelines of the *Photodiagnosis and Photodynamic Therapy* and *Journal of Photochemistry and Photobiology B: Biology*, respectively.

- ARTICLE 1 – Cytotoxic effect and apoptosis pathways activated by methylene blue-mediated photodynamic therapy in fibroblasts

- ARTICLE 2 – In vitro effect of curcumin-mediated antimicrobial photodynamic therapy on fibroblasts: viability and cell signaling for apoptosis

3 CONCLUSIONS

3 CONCLUSIONS

Based on the results, we can conclude that:

- High concentrations of aPDT mediated by methylene blue and curcumin promoted significantly reduction of viable fibroblasts when compared to control;
- In lower concentrations, MB- and curcumin-mediated aPDT did not promoted significant cellular cytotoxicity to mouse fibroblasts;
- MB- and curcumin-mediated aPDT increased the expression of Bax/Bcl-2 ratio, VDAC-1 and cytochrome C genes, suggesting the activation of an intrinsic apoptotic pathway.

4 REFERENCES

REFERENCES

ABRAHAMSE H, HAMBLIN MR. New photosensitizers for photodynamic therapy. Biochem. J. v. 473, p. 347–64, 2016.

AGGARWAL BB, KUMAR A, BHARTI AC. Anticancer potential of curcumin: preclinical and clinical studies, Anticancer Res. v. 23, p.363–98, 2003.

ANDRADE M et al. Effect of different pre-irradiation times on curcumin-mediated photodynamic therapy against planktonic cultures and biofilms of Candida spp. Arch Oral Biol. v. 58, n. 2, p. 200-10, 2012.

ANTO R et al. Curcumin (diferuloylmethane) induces apoptosis through activation of caspase-8, BID cleavage and cytochrome c release: Its suppression by ectopic expression of Bcl-2 and Bcl-xl. Carcinogenesis v. 23, p. 143–150, 2002.

ARAÚJO N et al. Photodynamic antimicrobial therapy of curcumin in biofilms and carious dentine. Lasers Med Sci. v. 29, p. 629-35, 2014.

ARAÚJO N et al. Photodynamic effects of curcumin against cariogenic pathogens. Photomed Laser Surg. v. 30, n. 7, p. 393-9, 2012.

BACELLAR IOL et al. Photodynamic Efficiency: From Molecular Photochemistry to Cell Death. Int. J. Mol. Sci. 2015, 16, 20523-20559; doi:10.3390/ijms160920523

BONSOR SJ et al. Microbiological evaluation of photo-activated disinfection in endodontics (an in vivo study). Br. Dent. J. v. 25, p. 337-41, 2006. [PubMed: 16568063]

BULIT F et al. Antimicrobial activity and cytotoxicity of 3 photosensitizers activated with blue light. J Endod. v. 40, p. 427-31, 2014.

BUNTING JR. A test of the singlet oxygen mechanism of cationic dye photosensitization of mitochondrial damage. Photochem Photobiol.v. 55, p. 81-7, 1992.

CHIAVIELLO A et al. Targets and mechanisms of photodynamic therapy in lung cancer cells: a brief overview, Cancers 3 p. 1014–41, 2011.

CHIGNELL CF et al. Spectral and photochemical properties of curcumin. Photochem Photobiol. v. 59, n. 3, p. 295-302, 1994.

CHRISTODOULIDES N et al. Photodynamic therapy as an adjunct to non-surgical periodontal treatment: a randomized, controlled clinical trial. J Periodontol. v. 79, n. 9, p. 1638-44, 2008.

CRIVELLO JV, BULUT U. Curcumin: a naturally occurring long-wavelength photosensitizer for diaryliodonium salts, J. Polym. Sci. Part A: Polym. Chem. v. 43, n. 21, p. 5217-31, 2005. https://doi.org/10.1002/pola.21017.

DAHL TA et al. Photokilling of bacteria by the natural dye curcumin. Arch Microbiol. v. 151, n. 2, p. 183-5,1989.

dos SANTOS AF et al. Methylene blue photodynamic therapy induces selective and massive cell death in human breast cancer cells. BMC Cancer. v. 17, n.1, 194, 2017. doi:10.1186/s12885-017-3179-7

DOVIGO L et al. Curcumin-mediated photodynamic inactivation of Candida albicans in a murine model of oral candidiasis. Med Mycol. v. 51, n. 3, p. 243-51, 2013.

DOVIGO L.N et al. Investigation of the photodynamic effects of curcumin against Candida albicans. Photochem Photobiol. v. 87, n. 4, p. 895-903, 2011.

DOVIGO, LN et al. Susceptibility of clinical isolates of Candida to photodynamic effects of curcumin. Lasers Surg Med. v. 43, n. 9, p. 927-34, 2011a.

DUVOIX A et al. Chemopreventive and therapeutic effects of curcumin, Cancer Lett. v. 223, n. 2, p. 181-90, 2005. https://doi.org/10.1016/j.canlet.2004.09.041.

ELMORE S. Apoptosis: a review of programmed cell death. Toxicol Pathol. v. 35, n. 4, p, 495-516, 2007.

FOSCHI F et al. Photodynamic inactivation of Enterococcus faecalis in dental root canals invitro. Lasers Surg Med. v. 39, p.782-7, 2007. [PubMed: 18081066]

GAJATE C, GONZALEZ-CAMACHO F, MOLLINEDO F. Lipid raft connection between extrinsic and intrinsic apoptotic pathways, Biochem. Biophys. Res. Commun. v. 380, n. 4, p. 780-84, 2009. https://doi.org/10.1016/j.bbrc.2009.01.147.

GEORGE S, KISHEN A. Advanced noninvasive light-activated disinfection: assessment of cytotoxicity on fibroblasts versus antimicrobial activity against Enterococcus faecalis. J Endod v. 33, p. 599-602, 2007. [PubMed: 17437881]

GHARESI S et al. Effect of photodynamic therapy based on indocyanine green on expression of apoptosis-related genes in human gingival fibroblast cells. Photodiagn. Photodyn. Ther. v. 19, p, 33-6, 2017.

GURSOY H et al. Photodynamic therapy in dentistry: a literature review. Clin. Oral Investig. v. 17, p, 1113–25, 2013. doi: 10.1007/s00784-012-0845-7

KASHEF N, ABADI GRS, DJAVID GE. Photodynamic inactivation of primary human fibroblasts by methylene blue and toluidine blue O. Photodiag Photodyn Ther. v. 9, p. 355-58, 2012. doi: 10.1016/j.pdpdt.2012.05.001.10.1016/j.pdpdt.2012.05.001

KESSEL D, LUO Y. Mitochondrial photodamage and PDTinduced apoptosis. J Photochem Photobiol B: Bio. v. 42, p. 89-95, 1998.

KONOPKA K, GOSLINSKI T. Photodynamic therapy in dentistry. J Dent Res. v. 86, n. 8, p. 694-704, 2007.

KUNWAR A et al. Quantitative cellular uptake, localization and cytotoxicity of curcumin in normal and tumor cells. Biochim Biophys Acta. v.1780, p. 673–79, 2008.

LEE JH et al. Involvement of both mitochondrial- and death receptor-dependent apoptotic pathways regulated by Bcl-2 family in sodium fluoride-induced apoptosis of the human gingival fibroblasts. Toxicology v. 243, p. 340-47, 2008.

LI H et al. Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. Cell. v. 94, n. 4, p. 491-501, 1998.

MAISCH, T. "Anti-microbial photodynamic therapy: useful in the future?," Lasers Med. Sci. v. 22, n. 2, p. 83-91, 2007.

NASTRI L et al. Effects of toluidineblue-mediated photodynamic therapy on periopathogens andperiodontal biofilm: in vitro evaluation. Int J. Immuno Pathol. Pharmacol. v. 23, n. 4, p. 1125-32, 2010.

NIKAIDO H. Molecular basis of bacterial outer membrane permeability revisited. Microbiol Mol Biol Rev. v. 67, n. 4, p. 593–656, 2003.

NOODT BB et al. Apoptosis induction by different pathways with methylene blue derivative and light from mitochondrial sites in V79 cells. Int J Cancer. v.75, p. 941-48, 1998.

OLIVEIRA CS et al. Major determinants of photoinduced cell death: Subcellular localization versus photosensitization efficiency. Free Radic Biol Med. v. 51, n.4, p. 824–33, 2011.

OLIVEIRA FA et al. Low intensity lasers differently induce primary human osteoblast proliferation and differentiation, J. Photochem. Photobiol. B v. 163, p. 14–21, 2016.

PASCHOAL MA et al. Photodynammic potential of curcumin and blue LED agaisnt Streptococcus mutans in a planktonic culture. Photodiag Photodyn Ther. v. 10, n. 3, p. 313-19, 2013.

PILEGGI, G. et al. Blue light-mediated inactivation of Enterococcus faecalis in vitro. Photodiagnosis Photodyn Ther. v. 10, n. 2, p. 134-40, 2013.

QIAO J et al. Photodynamic effects on human periodontal-related cells in vitro, Photodiag. Photodyn. Ther. v. 11, n. 3, p. 290-99, 2014 https://doi.org/10.1016/j.pdpdt.2014.04.001.

RIBEIRO APD et al. Photodynamic therapy associating Photogem® and blue LED on L929 and MDPC-23 cell culture. Cell Biol. Int. v. 34, p. 343-351, 2010.

RIBEIRO APD et al. Phototoxic effect of curcumin on methicillin-resistant Staphylococcus aureus and L929 fibroblasts, Lasers Med. Sci. v. 28, p. 391-98, 2013. doi 10.1007/s10103-012-1064-9.

RIEDL SJ, SALVESEN GS. The apoptosome: signalling platform of cell death. Nat. Rev. Mol. Cell Biol. v. 8, p. 405-13, 2007.

ROSA LP et al. Antimicrobial photodynamic inactivation of Staphylococcus aureus biofilms in bone specimens using methylene blue, toluidine blue ortho and malachite green: an in vitro study. Arch. Oral Biol. v. 60, p. 675–80, 2015.

ROVALDI CR et al. Photoactive porphyrin derivative with broad-spectrum activity against oral pathogens in vitro. Antimicrob. Agents Chemother. v. 44, n. 12, p. 3364-7, 2000.

SHOSHAN-BARMATZ V, DE S, MEIR A. 2017. The Mitochondrial Voltage-Dependent Anion Channel 1, Ca(2+) Transport, Apoptosis, and Their Regulation. Front Oncol v. 7, p. 60, 2017

SOUKOS NS et al. Photodynamic therapy for endodontic disinfection. J. Endod. v.32, p. 979-84, 2006. [PubMed: 16982278]

STEINER-OLIVEIRA CS et al. Randomized in vivo evaluation of photodynamic antimicrobial chemotherapy on deciduous carious dentin. J. Biomed. Optics. v. 20, n. 10, p. 108003, 2015.

TARASZKIEWICZ A et al. Innovative strategies to overcome biofilm resistance. Biomed. Res. Int. v. 2013, ID 150653, 13 pages, 2013.

TARDIVO JP et al. Methylene blue in photodynamic therapy: From basic mechanisms to clinical applications. Photodiagnosis Photodyn Ther. v. 2, p. 175–91, 2005.

THOMAS CD et al. Cellular density, a major factor involved in PDT cytotoxic responses: study on three different lines of human retinoblastoma grafted on nude mice. Photodiag. Photodyn. Ther. v. 12, p. 267-75, 2015.

TUITE EM, KELLY JM. Photochemical interactions of methylene blue and analogues with DNA and other biological substrates. J Photochem Photobiol B. v. 21, p. 103-24, 1993.

VATANSEVER F et al. Antimicrobial strategies centered around reactive oxygen species- bactericidal antibiotics, photodynamic therapy, and beyon. FEMS Microbiol. Rev. v. 37, n. 6, p. 955-89, 2013. doi: 10.1111/1574-6976.12026

WOO J et al. Molecular mechanisms of curcumin-induced cytotoxicity: Induction of apoptosis through generation of reactive oxygen species, down-regulation of Bcl-XL and IAP, the release of cytochrome c and inhibition of Akt. Carcinogenesis v. 24, p. 1199-1208, 2003.