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GABRIELA BESSA MARCONATO ANTUNES

EFEITOS DA LIDOCAÍNA LIVRE E COMPLEXADA COM 2-HIDROXIPROPIL-β-CICLODEXTRINA SOBRE A VIABILIDADE E PROLIFERAÇÃO DO CARCINOMA DE CÉLULAS ESCAMOSAS

EFFECTS OF PLAIN LIDOCAINE AND LIDOCAINE COMPLEXED WITH 2-HYDROXYPROPYL-β-CYCLODEXTRIN ON VIABILITY AND PROLIFERATION OF ORAL SQUAMOUS CELL CARCINOMA

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Trabalho de Conclusão de Curso apresentado à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dos requisitos exigidos para obtenção do título de Cirurgiã Dentista.

Undegraduate final work presented to the Piracicaba Dental School of the University of Campinas in partial fulfillment of the requirements for the degree of Dental Surgeon.

Orientador: Profa. Dra. Maria Cristina Volpato

Coorientador: Prof. Dr. Luiz Eduardo Nunes Ferreira

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RESUMO

O carcinoma de células escamosas (CCE) é o tumor mais prevalente da cavidade oral. A lidocaína tem demostrado atividade antiproliferativa e citotóxica em vários tipos celulares, porém sua utilização é limitada pela rápida dispersão. Carreadores como as ciclodextrinas podem aumentar a biodisponibilidade e diminuir a toxicidade de fármacos. Este estudo avaliou os efeitos da lidocaína livre (lido) e complexada com 2-hidroxipropil-β-ciclodextrina (HP-β-CD-lido) sobre a viabilidade celular (VC) e proliferação de células (PC) de carcinoma de células escamosas de língua humana SCC9 e SCC25. Estas células foram expostas a lido e HP-β-CD-lido, com concentração variando de 40 a 4000 µM. VC foi avaliada pelo teste de redução do MTT e PC pelo método da sulforrodamina B. Células não tratadas e tratadas com doxorrubicina constituíram os grupos controle negativo e positivo, respectivamente. Os dados foram submetidos à análise de regressão não linear (α=5%). Ambas formulações afetaram a VC e a PC das células SCC9 e SCC25. O efeito citotóxico da lidocaína foi potencializado pela HP-β-CD-lido (4000 µM) nas duas linhagens celulares. Lido e HP-β-CD-lido (4000 µM) reduziram ($p<0,05$) a viabilidade das células SCC9 para 83% e 63%, respectivamente, e da linhagem SCC25 para 71% e 44%, respectivamente. Lido (4000 µM) reduziu a proliferação de SCC9 e SCC25 para 39,5% e 23,7%, respectivamente. A doxorrubicina diminuiu VC e PC nas duas linhagens em concentração inferior às de lido e HP-β-CD-lido ($p<0,001$). A lidocaína demonstrou promover benefícios terapêuticos ao tratamento do carcinoma de células escamosas e a HP-β-CD-lido potencializou a citotoxicidade da lidocaína.

Palavras-chave: 2-Hidroxipropil-β-ciclodextrina; Lidocaína; Carcinoma de células escamosas.

ABSTRACT

Squamous cell carcinoma (SCC) is the most prevalent tumor in the oral cavity Lidocaine has been shown antiproliferative and cytotoxic effects on various cell lines. Its use is limited by its rapid dispersion to the tissues. Drug carriers such as cyclodextrins can improve bioavailability and reduce drug toxicity. This study evaluated the effects of plain lidocaine (lido) and lidocaine complexed with 2-hydroxypropyl-β-cyclodextrin (HP-β-CD-lido) on squamous cell carcinoma SCC9 and SCC25 viability (CV) and proliferation (CP). These cells were exposed to lido and HP-β-CD-lido, in concentrations ranging from 40 to 4000 µM. CV was evaluated by MTT reduction test and PC by sulforhodamine B test. Negative and positive control groups were, respectively, non treated cells and cells treated with doxorubicin. The results were submitted to non linear regression (non fit) (α=5%). Both formulations reduced viability and proliferation of SCC9 and SCC25 cells. The cytotoxic effect of lido was potentialized by HP-β-CD-lido (4000 µM) in both cell lines. The viability was reduced ($p<0.05$) by lido and HP-β-CD-lido (4000 µM) to 83% and 63% for SCC9, and to 71% and 44%, for SCC25, respectively. Lido (4000 µM) reduced proliferation of SCC9 to 39.5% and of SCC25 to 23.7%. Doxorubicin reduced CV and CP of both cell lines at lower concentrations in comparison to lido and HP-β-CD-lido ($p<0.001$). Lidocaine may provide therapeutic improvement to the treatment of squamous cell carcinoma; the complexation with HP-β-CD potentialized lidocaine effects.

Keywords: 2-hydroxypropyl-β-ciclodextrin; Lidocaine; Squamous cell carcinoma.

SUMÁRIO

1 INTRODUÇÃO

A ocorrência de tumores na cavidade oral é frequente, situando-se entre os dez locais mais afetados do organismo. De acordo com o Instituto Nacional do Câncer (INCA, 2018), a estimativa de novos casos de câncer na cavidade oral é de 10,86 a cada 100 mil entre os homens e de 3,28 a cada 100 mil entre as mulheres, ocupando a quinta e a décima segunda posições de ocorrência, respectivamente, para cada gênero.

Dentre os tumores incidentes na cavidade oral, o mais comumente encontrado, o carcinoma de células escamosas (CCE) ocorre em proporção acima de 90% (WARNAKULASURIYA, 2009), sendo a porção lateral da língua o local mais afetado (Nóbrega et al., 2018).

Entre os fatores de risco para a doença incluem-se infecções por HPV, radiação solar e uso de tabaco e álcool, não podendo ser negligenciados os fatores genéticos (Brener et al., 2007; Andrews et al., 2009). O tamanho do tumor e a ocorrência de metástases nos linfonodos cervicais são fatores importantes, que afetam negativamente o prognóstico (Iype et al., 2008; Nóbrega et al., 2018).

A escolha do tratamento para o CCE depende do estadiamento da doença e inclui, na maioria dos casos, a cirurgia (incluindo ou não a dissecção do pescoço, visando eliminar possíveis metástases nos linfonodos desta região), seguida ou não de quimioterapia ou radioterapia (Brener et al., 2007; Ding et al., 2018). A taxa de sobrevivência de pacientes diagnosticados com CCE tem se mantido constante nos últimos 30 anos, ao redor de 50% (Nóbrega et al., 2018) ou abaixo deste valor (Le Campion, 2017), o que mostra a necessidade de técnicas e medicamentos alternativos na abordagem desta doença.

Neste sentido, têm sido estudados os efeitos antiproliferativos e pró-apoptóticos de compostos de origem natural (Hoornstra et al., 2018) e, ainda, de substâncias sintéticas, como os anestésicos locais (Zhang et al., 2017). Estes últimos constituem o grupo de medicamentos mais utilizado na odontologia e em cirurgia regional. São utilizados na remoção de tumores de cabeça e pescoço (Kobayashi et al., 2012), e seu uso tem sido associado à redução da incidência de metástases (Wada et al., 2007) e aumento da sobrevida, o que poderia estar relacionado aos efeitos

secundários promovidos pela ação anti-inflamatória, diminuindo assim a proliferação e migração das células tumorais (Chamaraux-Tran & Piegeler, 2017).

A lidocaína, um anestésico local classificado como amino-amida, tem demonstrado efeitos inibitórios sobre vários tipos de células tumorais (Sakaguchi et al., 2006; Perez-Castro et al., 2009; Chamaraux-Tran, 2017; Zhang et al., 2017; Bundscherer et al., 2017), além de potencializar o efeito de fármacos usados no tratamento de tumores, como relatado por Yang et al. (2018), em tumor da bexiga urinária. De importância clínica é o fato de que os efeitos antitumorais exercidos pela lidocaína são obtidos com concentrações normalmente utilizadas em procedimentos cirúrgicos (Mammoto et al., 2002).

Entretanto, por permanecer por curto período de tempo no local injetado, sua ação é consideravelmente curta quando comparada a anestésicos de ação longa, como a bupivacaína. Na obtenção de bloqueio de fibras sensitivas para promoção de anestesia ou para controle de dor, seu tempo de ação pode ser aumentado por vasoconstritores (Andrade, 2014). Além dos vasoconstritores, também têm sido estudadas substâncias carreadoras de drogas, as quais permitem melhorar as propriedades farmacocinéticas e biofarmacêuticas (de Paula et al., 2010).

Dentre os carreadores de drogas, as ciclodextrinas tem se mostrado bastante promissoras. Ao promover a liberação mais lenta dos fármacos, as ciclodextrinas contribuem para diminuir sua toxicidade, além de melhorar a biodisponibilidade (de Araujo et al., 2007). Em acréscimo a estabilização de fármacos (como a cetirizina, por exemplo) e o aumento da solubilidade promovida pelas ciclodextrinas também pode contribuir para o aumento da biodisponibilidade (Paczkowska et al., 2018).

As ciclodextrinas são compostos cíclicos obtidos a partir da hidrólise do amido, os quais podem apresentar seis, sete ou oito unidades de glicose unidas por ligações α-1,4. De acordo com a quantidade de unidades de glicose as ciclodextrinas são classificadas em α (seis unidades), β (sete unidades) e γ (oito unidades). Apresentam a forma de um semi-cone ou cone truncado, sendo o seu interior hidrofóbico e o exterior hidrofílico; desta forma, conseguem carrear drogas com caráter hidrofóbico, aumentando sua solubilidade em água (Mura, 2014).

As β-ciclodextrinas tem atraído interesse por serem menos tóxicas que as demais e foram liberadas para uso oral e por via infiltrativa pela agência que regula medicamentos e alimentos nos Estados Unidos, a Food and Drug Administration - FDA (de Paula et al., 2012). Estas ciclodextrinas têm sido utilizadas para complexar diversos tipos de fármacos (Dollo et al., 1998; Liu et al., 2006; de Araújo et al., 2007; Eida et al., 2011; Ma et al., 2012; Liu et al., 2014; Wang et al., 2014; Han et al., 2014; Zhang et al., 2016).

Foi demonstrado por Moraes et al. (2007) que a 2-hidroxipropil-β-ciclodextrina é eficaz em formar complexo de inclusão estável com a lidocaína, além de apresentar alta hidrossolubilidade. Os resultados deste estudo, aliados aos daqueles que demonstraram efeito antitumoral da lidocaína, levaram à hipótese de que a lidocaína poderia exercer este mesmo efeito contra o carcinoma de células escamosas e de que a complexação da lidocaína com 2-hidroxipropil-β-ciclodextrina poderia aumentar este efeito.

Desta forma, este projeto buscou avaliar os efeitos in vitro da lidocaína livre e no complexo de inclusão 2-hidroxipropil-β-ciclodextrina/lidocaína sobre a viabilidade celular e atividade antiproliferativa em células de carcinoma de células escamosas de língua humano das linhagens SCC9 e SCC25.

2 ARTIGO: EFFECTS OF LIDOCAINE AND THE INCLUSION COMPLEX WITH 2-HYDROXYPROPYL-B-CYCLODEXTRIN ON CELL VIABILITY AND PROLIFERATION OF ORAL SQUAMOUS CELL CARCINOMA

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O estudo desenvolvido por Gabriela Bessa Marconato Antunes para o presente Trabalho de Conclusão de Curso constitui a parte do artigo referente à cultura de células SCC9 e SCC25 e avaliação das formulações de lidocaína livre e no complexo de inclusão 2-hidroxipropil-β-ciclodextrina/lidocaína sobre a viabilidade celular e atividade antiproliferativa destas células.

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Research Paper

Effects of lidocaine and the inclusion complex with 2-hydroxypropyl-β-cyclodextrin on cell viability and proliferation of oral squamous cell carcinoma

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Keywords
2-hydroxypropyl-β-cyclodextrin; anticancer activity; lidocaine; squamous cell carcinoma

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Abstract

Objectives Squamous cell carcinoma (SCC) is a malignant disease that affects the oral cavity. Lidocaine has shown antiproliferative and cytotoxic activity on several cell types. The rapid dispersion is a limitation issue; however, the complexation in cyclodextrin improved pharmacological features and modified the drug release. This study investigated the effects of lidocaine (lido) complexed with 2-hydroxypropyl-β-cyclodextrin (HP-β-CD-lido) on cell viability and proliferation of human tongue squamous cell carcinoma SCC9 and SCC25.

Methods The complex formation was confirmed by differential scanning calorimetry (DSC) and scanning electron microscopy (SEM). Cells SCC9 and SCC25 were exposed to lido and HP-β-CD-lido (40–4000 μM), and the effects on cell viability (MTT) and antiproliferative activity (SRB) were tested.

Key findings Differential scanning calorimetry and SEM results demonstrated the occurrence of host–guest interaction. Lido and HP-β-CD-lido (4000 μM) significantly reduced the viability of SCC9 cells to 83% and 63%, respectively. The viability of SCC25 treated with lido, and HP-β-CD-lido (4000 μM) was 71% and 44%, respectively. Lido (4000 μM) reduced the proliferation of SCC9 and SCC25 to 39.5% and 23.7%, respectively. HP-β-CD-lido (4000 μM) was cytotoxic for both cell lines.

Conclusions HP-β-CD was able to potentiate the *in vitro* cytotoxic effects of lidocaine on human squamous cell carcinoma.

Introduction

Squamous cell carcinoma (SCC) represents about 90% of all malignancies in the oral cavity.[1] The lower lip is the most common anatomical site for oral SCC. In the intraoral region, the lateral border of the tongue has higher incidence rates.[2,3] The occurrence of oral squamous cell carcinoma (OSCC) in the tongue can lead to metastasis to regional lymph nodes, which is one of the most important prognoses for patient survival.[4,5] In an attempt to reduce OSCC mortality, new surgical techniques and therapeutic strategies with chemotherapeutic agents have been developed.[6] Local anaesthetics (LA) may be useful during the treatment of OSCC. The amphiphilic nature of LA allows the distribution across the cellular membranes, modulating several cellular pathways even in lower concentrations than those required to block sodium channels.[7,8]

Local anaesthetics are often administered in the adjacent tissue during tumour surgeries in the head and neck region.[9] The use of LA has been shown to reduce metastasis and tumour reoccurrence.[10] Lidocaine is an amino amide-type local anaesthetic most commonly used in medical and dental practice. It has been able to inhibit the invasion of cancer cells at the concentrations used in surgical

procedures.[11] The topical use of lidocaine for pain relief can also reduce the proliferation of cancer cells in tongue tumour.[12]

However, the therapeutic action of lidocaine is limited by a short time of action, due to its fast dispersion from the injection site.[13] The association with drug delivery systems allows the manipulation of pharmacological and biopharmaceutical properties of LA.[14,15]

Cyclodextrins (CD) are able to form inclusion complexes with a wide variety of molecules, leading to modified drug release, changes in solubility and other physicochemical properties.[16] Cyclodextrins are cyclic oligosaccharides produced by enzymatic hydrolysis of the starch, constituted by six (α-CD), seven (β-CD) or eight (γ-CD) D-glucose monomers linked by α-1,4-glucose bonds.[17] The shape of cyclodextrin molecules resembles truncated cones, with a hydrophobic internal cavity and a hydrophilic outer surface.[18]

The complexation of LA in cyclodextrins provides pharmacological improvements and therapeutic benefits, by slowing the drug release, increasing anaesthetic duration, and reducing its toxicity.[19,20] The 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) is a hydroxy-alky lated β-CD derivative with excellent aqueous solubility and satisfactory inclusion capacity, which has demonstrated the ability to form a stable complex with lidocaine.[21,22]

The main hypothesis of this study is that lidocaine exerts cytotoxic effects on oral squamous cell carcinoma. This effect is probably improved by its complexation with HP-β-CD. Thus, this study aimed to evaluate the *in vitro* effects of the lidocaine and lidocaine complexed with HP-β-CD (HP-β-CD-lido) on the cell viability and proliferation of two human tongue squamous cells carcinoma (SCC9 and SCC25).

Materials and Methods

Reagents

Lidocaine hydrochloride, Dulbecco's modified Eagle's medium/Nutrient Mixture F-12 Ham (DMEM/F12), glutamine, sulforhodamine B, hydrocortisone, HEPES buffer, penicillin-streptomycin and gentamicin were purchased from Sigma-Aldrich (St. Louis, MO, USA). The 2-Hydroxypropyl-β-cyclodextrin was purchased from Roquettes Serv. Tech. Lab (Lestrem, France), and doxorubicin was obtained from Cayman Chemical (Ann Arbor, MI, USA). Fetal bovine serum (FBS), 0.25% trypsin with 0.5 mM EDTA, trypan blue and MTT salt (1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan) were purchased from Life Technologies (Carlsbad, CA, USA).

Preparation of the HP-β-CD-lido inclusion complex (HP-β-CD-lido)

The solid inclusion complex was prepared following the protocols proposed by Dollo *et al.*[23] and Loftsson & Masson.[24] Briefly, 2-hydroxypropyl-β-cyclodextrin (HP-β-CD) and the lidocaine hydrochloride (lido) were added to deionized water in a 1 : 1 molar ratio. The solution was shaken for 24 h at room temperature to reach equilibrium, followed by freeze-drying lyophilization and storage at −20°C. HP-β-CD-lido complex was resuspended in DMEM/F12 medium supplemented with FBS and antibiotics for the cytotoxicity assays. Before use, the solution was sterilized by filtration in membranes with 0.22 μm pore (TPP).

Differential scanning calorimetry (DSC)

The DSC method is based on the determination of the heat involved in the exothermic and endothermic transitions.[25] DSC analysis was performed following the protocol of Silva *et al.*[26] The sample was tested in a DSC Q20 (TA Instruments, New Castle, DE, USA) equipped with a cooling system, under a nitrogen flux of 50 ml/min and 10°C/min and a heating rate ranging from 5 to 400°C. Indium was used to calibrate the temperature, and 5 mg of the lido, HP-β-CD, HP-β-CD-lido or the physical mixture of lido and HP-β-CD were placed in aluminium pans at the sample compartment. An empty pan served as reference.

Scanning electron microscopy (SEM)

To evaluate possible structural changes in the crystals after complexation, freeze-dried samples of lido, HP-β-CD, HP-β-CD-lido and the physical mixture were fixed on aluminium stubs with double-sided carbon tape. The samples were metallized with gold in a sputter coater (Bal-Tec SCD050 Sputter Coater, SP, Brazil) under a vacuum for 120 s. Images were analysed in the Scanning Electron Microscope (Jeol, JSM 5800LV, Japan) in the secondary electrons mode with 15 kV acceleration.

Cell culture

Immortalized human tongue squamous carcinoma cells from SCC9 and SCC25 lines were cultured in DMEM/F12 medium supplemented with 10% FBS and 400 ng/ml hydrocortisone, penicillin (100 U/ml), streptomycin (100 μg/ml) and gentamycin (10 μg/ml). Cells were grown in tissue culture plates (61 cm^2) under humidified atmosphere at 5% CO_2/95% air, 37°C. Treatment with 0.25% trypsin and 0.5 μM EDTA was used to transfer the cells to the 96-well tissue plates or to duplicate the cell culture.

Experiments were performed using approximately 80% of confluence.

Experimental conditions

SCC9 and SCC25 cells were washed with prewarmed PBS (37°C) and detached with 0.25% trypsin and 0.5 μM EDTA. Then, 5×10^4 cells/ml were transferred to the 96-well tissue plates and incubated at 37°C in 5% CO_2 for 24 h. Cells were exposed to treatments with lido or HP-β-CD-lido diluted in supplemented DMEM/F12 at concentrations ranging from 40 to 4.000 μM.[12] The exposition periods for cell viability and antiproliferative assays were 24 and 48 h, respectively. Doxorubicin hydrochloride was used as positive control at concentrations of 0.043 to 430 μM, equivalent to 0.025 to 250 μg/ml.[27] Negative control included untreated cells grown in the supplemented DMEM/F12. The effects of 4000 μM HP-β-CD without lidocaine were also tested; all experiments were performed in triplicates.

Cell viability assay

After incubation with lidocaine formulations, the cell viability was measured by MTT reduction. Briefly, the wells were washed with prewarmed PBS (pH 7.4), and then 200 μl of DMEM/F12 with 0.5 mg/ml MTT sodium salt was added. The plates were incubated for 4 h to MTT reduction. The medium was removed, the cells were washed with PBS again, and 100 μl of absolute ethanol was added to each well to dissolve the formazan crystals. Optical density was measured using a microplate reader set at 450 nm wavelength.

Effects on cell proliferation by the sulforhodamine B test (SRB)

The assays were performed according to the model for anticancer drugs developed by National Cancer Institute NCI/NIH (Frederick, WA, USA). Cells were grown as previously described and exposed to lido, HP-β-CD-lido and doxorubicin formulations. Part of the cells was fixed with 10% trichloroacetic acid (TCA), forming a control group time 0 (T_0) without cell growth. The plates were incubated for 48 h at 37°C. Then, the cells were fixed with 10% TCA and stained with 4% SRB in 1% acetic acid. The SRB bound to the cells was solubilized with 150 μl of 10 μM Triz base (pH 10.5). The absorbance was measured in a microplate reader set at 515 nm wavelength.

The absorbance values from time 0 (T_0), control group (C) and treatments (Tx) were used to calculate the percentage of cell growth with the formula: $[(1 - (Tx - T_0)/(C - T_0)) \times 100\%]$.[28]

Statistical analysis

All of the data was tested for normal distribution (Shapiro–Wilks' test) and variance homoscedasticity (Levene's test). The data was analysed using the analysis of variance (ANOVA) followed a post hoc analysis by Tukey's test. The *t*-test was applied to the *in vitro* release data. Nonlinear regression analysis (nonlin fit) was used to observe the cell viability and proliferation. All analyses were performed using the Biostat® 5.0 (Mamirauá Institute, PA, Brazil) and GraphPad Prism 6.0 (GraphPad Software, Inc., La Jolla, CA, USA) software. Significance level was set at 5% ($\alpha = 0.05$).

Results

Differential scanning calorimetry

Representative DSC thermograms related to the rate of heat absorbed by lido, HP-β-CD, HP-β-CD-lido

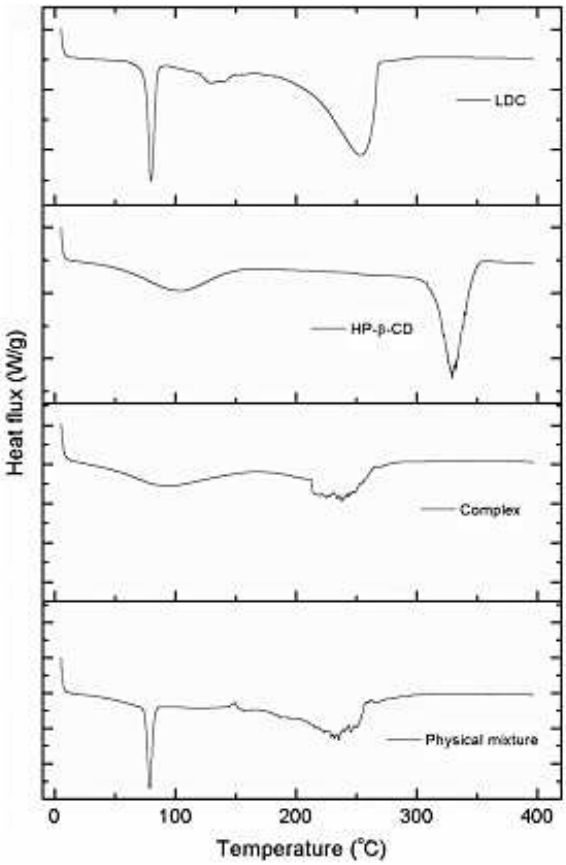

Figure 1 DSC thermograms of lidocaine (LDC), 2-hydroxypropyl-β-cyclodextrin (HP-β-CD), HP-β-CD-lido (Complex) and physical mixture of lido and HP-β-CD (Physical mixture). DSC, differential scanning calorimetry.

complex and the physical mixture are shown in Figure 1. Lido showed an endothermic peak at 80.2°C, corresponding to the melting point of this drug and another peak at 252.3°C related to thermal degradation. HP-β-CD presented large endothermic peak with maximum at 101.4°C, indicating the release of the water molecule present in the CD cavity and another characteristic endothermic peak at 330.2°C corresponding to the HP-β-CD melting point.[29]

The thermogram of the physical mixture shows two endothermic peaks related to lido and HP-β-CD molecules. In contrast, these peaks are absent in the thermogram of the complex (HP-β-CD) indicating that lidocaine is present in the HP-β-CD cavity. These results serve as evidence for the formation of HP-β-CD-lido complex, showing that the complexation method was adequate.

Morphology

Scanning electron microscopy (SEM) images of the arrangements and morphological aspects of lido, HP-β-CD, HP-β-CD-lido and physical mixture are shown in Figure 2. Figure 2a and 2b show typical crystals of lidocaine and HP-β-CD, which are found in different sizes, respectively. There are changes in the structure of the HP-β-CD -lido due to the interaction between the two molecules. The physical mixture revealed some similarities with the crystals of the free molecules. Thus, the SEM provides more evidence of the complex formation between lidocaine and HP-β-CD-lido as previously reported for ropivacaine.[19]

Effects of lidocaine formulations on cell viability

The effects of lidocaine formulations on the viability of SCC9 and SCC25 cells are presented in Figure 3. Nonlinear regression analysis (nonlin fit) showed that doxorubicin leads to a significant decrease ($P < 0.0001$) on cell viability in lower concentrations than the lidocaine formulations in both cells. HP-β-CD-lido significantly reduced cell viability in comparison with lidocaine in the SCC9 and SCC25 cells.

The SCC25 cells were more susceptible to the treatments. The half-maximal effective concentration 50% ($EC_{50\%}$) for doxorubicin in SCC9 and SCC25 cells were 3.21 μM (SEM ±0.22) and 0.88 μM (SEM ±0.037), respectively. Treatment with 4000 μM lido reduced the cell viability of SCC9 cells to 83%, while HP-β-CD-lido reduced the cell viability to 63% at the same concentration (Figure 3a). In SCC25 cells, 4000 μM lido reduced cell viability to 71%, and HP-β-CD-lido to 44% (Figure 3b). A treatment with 4000 μM HP-β-CD was also tested. Viability of SCC9 and SCC25 cells showed means of 91.6% and 93.6%, respectively. Thus, the increased cytotoxicity induced by the HP-β-CD-lido may relate to a synergism between the two molecules.

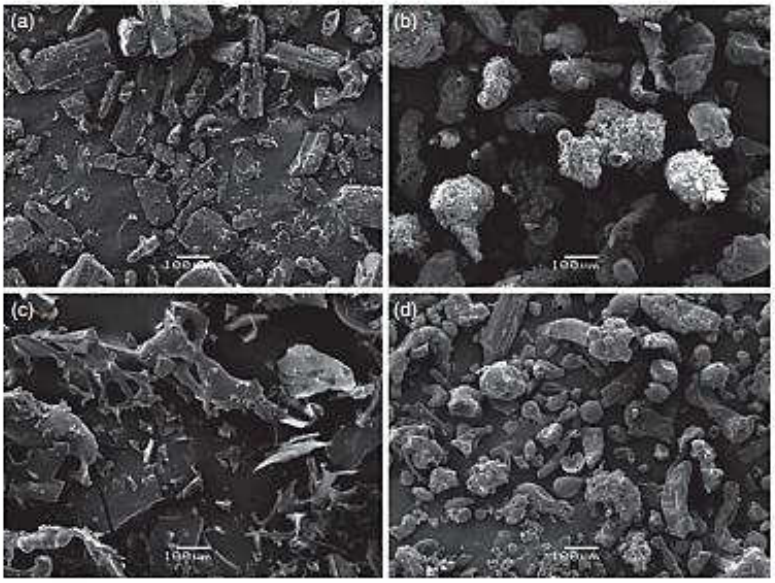

Figure 2 Scanning electron microscopy images of lidocaine (a), HP-β-CD (b), HP-β-CD-lidocaine (c) and physical mixture of lidocaine and HP-β-CD (d).

Effects of lidocaine formulations on cell proliferation

The results of the *in vitro* antiproliferative activity for SCC9 and SCC25 cells are shown in Figure 4. Doxorubicin showed the highest antiproliferative activity in both of the cell lines. HP-β-CD-lido significantly increased the antiproliferative activity in relation to lido for SCC9 ($P = 0.0090$) and SCC25 ($P < 0.0001$) cells. The half-maximal inhibitory concentration ($IC_{50\%}$) values are shown in Table 1.

Antiproliferative effects in SCC9 and SCC25 cells were observed at 2200 μM for both lidocaine formulations. Similar to the cell viability results, SCC25 showed to be more susceptible to the effects of the treatments on cell proliferation. The SCC9 proliferation was reduced to 39.5% after treatment with 4000 μM lido. HP-β-CD-lido at this concentration had a cytotoxic effect, in which the number of cells was reduced to a value below T0 (Figure 4a).

Treatments with lido at 2200 and 4000 μM reduced the SCC25 proliferation to 55.4% and 23.7%, respectively. HP-β-CD-lido at 2200 μm reduced SCC25 proliferation to 56.9%, and a cytotoxic activity was observed with 4000 μM HP-β-CD-lido (Figure 4b). A treatment with 4000 μM HP-β-CD did not show any difference in the SCC9 and SCC25 proliferation rate in comparison with control group.

Discussion

Local anaesthetics have a broad spectrum of pharmacological actions. Experimental studies in murine models of

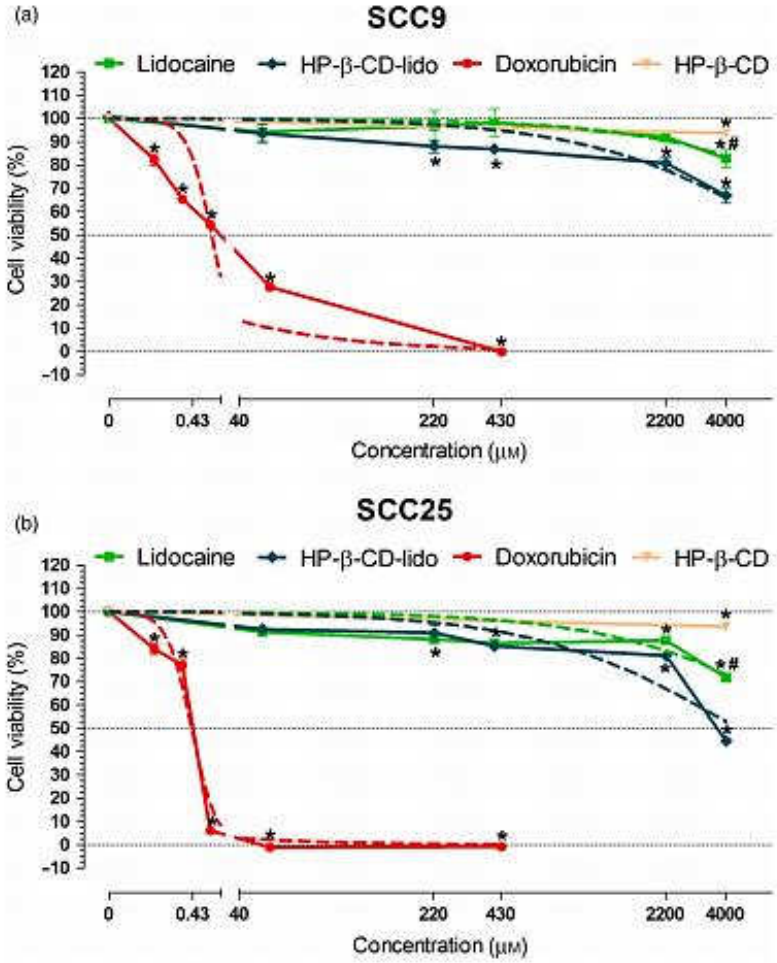

Figure 3 Viability (mean ± SEM) of SCC9 (a) and SCC25 (b) cells after 24 h exposure to different concentrations of lido, HP-β-CD-lido and doxorubicin. *Y*-axis = cell viability in % relative to the control group. *X*-axis = log of the drug concentration (μM). Nonlin fit (dotted line); $^{*}P < 0.01$ between treatments and the control group (100% cell viability); $^{\#}P < 0.01$ between 4000 μM lido and 4000 μM HP-β-CD-lido. [Colour figure can be viewed at wileyonlinelibrary.com]

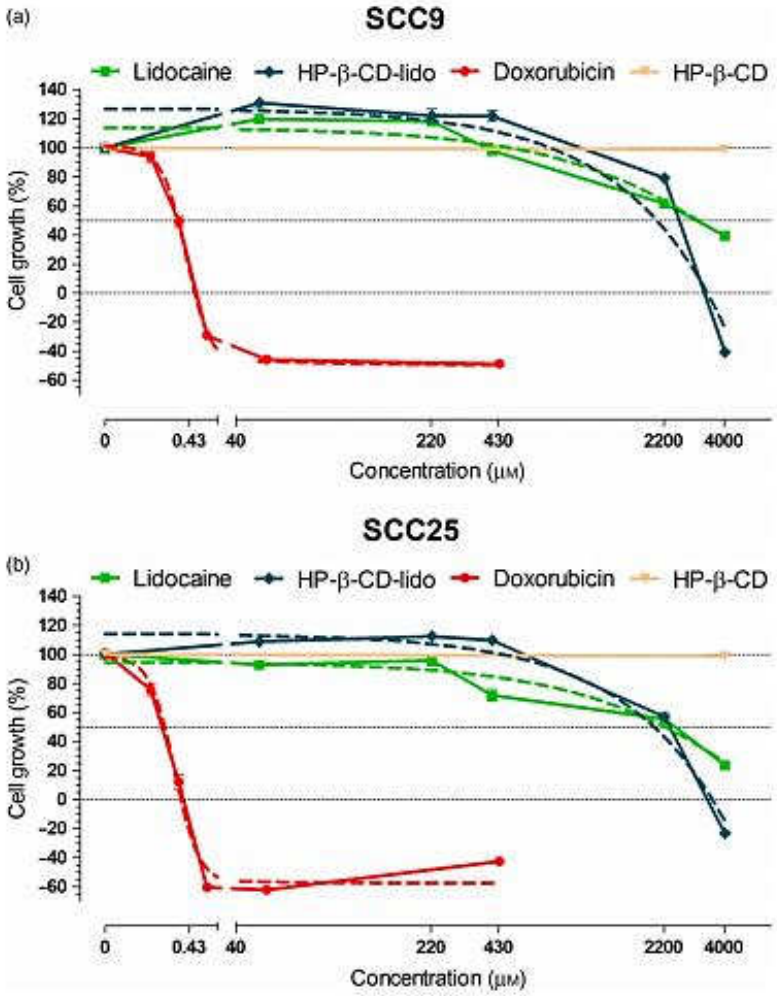

Figure 4 Antiproliferative effects (mean ± SEM) of lido, HP-β-CD-lido and doxorubicin on SCC9 (a) and SCC25 (b) cells after 48 h of exposition to the treatments. *Y*-axis = cell growth in % relative to the control group. *X*-axis = log of the drug concentration (μM). Nonlin fit (dotted line); $P = 0.009$ between lido and HP-β-CD-lido for SCC9 and $P < 0.0001$ for SCC25. [Colour figure can be viewed at wileyonlinelibrary.com]

Table 1 The half-maximal inhibitory concentration ($IC_{50\%}$ ± standard error) of doxorubicin, lido and HP-β-CD-lido on SCC9 and SCC25 cells proliferation after 48 h exposure to the treatments

$IC_{50\%}$ ± standard error				
Cell	Doxorubicin (μM)	Lido (μM)	HP-β-CD-lido (μM)	*P* (lido × HPβCD-lido)
SCC9	0.30 (±0.051)	3632 (±116.1)	1839 (±160.60)	$P = 0.009$
SCC25	0.086 (±0.020)	1782 (±48.93)	1540 (±88.81)	$P < 0.0001$

breast cancer showed metastasis reduction with the use of regional anaesthesia.[30,31] Moreover, the use of regional anaesthesia has shown benefits in patients undergoing cancer surgery.[7] Based on these pieces of evidence, we decided to study the effects of lidocaine and the inclusion complex HP-β-CD-lido on cell viability and proliferation in oral squamous cell carcinoma.

The DSC and SEM images confirmed the lidocaine inclusion into HP-β-CD molecules. When guest molecules are included inside cyclodextrin cavities, their melting, boiling

or sublimation point changes to a different temperature or disappears in the cyclodextrin decomposition range[29] which was observed in the HP-β-CD-lido thermogram. In addition, SEM images showed a structural alteration of the complex in comparison with lido, HP-β-CD and the physical mixture of these substances.

The complete characterization of the HP-β-CD-lidocaine complex has already been demonstrated in previous studies applying, in addition to DSC and SEM, other techniques such as the phase solubility study, power X-ray diffractometry, nuclear magnetic resonance, Fourier-transform infrared spectroscopy, *in vitro* release and others methods.[21,22] Drug delivery systems act as reservoirs delaying the release of the local anaesthetic. Thus, the complexation in HP-β-CD can enhance the pharmacological properties of lidocaine, avoiding its dispersion from the injected site.[14]

Lidocaine has shown cytotoxic activity in different cell lines *in vitro*.[32–34] In the present study, the effects of lidocaine formulations on viability (MTT) and proliferation (SRB) of SCC9 and SCC25 cells were evaluated. In tongue cancer cells (CAL27), 400 μM lidocaine suppressed cell proliferation after 72 and 120 h of exposure. Furthermore, 4000 μM lidocaine induced a reduction in cell viability after 24 h and cytotoxicity after 72 h.[12] In another study with OSCC cells from the HSC-2, HSC-3, HSC-4, NA and Ca9-22 lines, lidocaine had a CC_{50} (50% cytotoxic concentration) mean of 676.6 μM after exposure for 48 h.[9]

In general, these results are consistent with those observed in our study, in which lidocaine formulation exhibited relevant cytostatic and cytotoxic effects in the range of 2200 to 4000 μM. The differences observed among the studies can be attributed to methodological aspects such as the time of exposure and the susceptible profile of the cell lines.

In clinical practice, lidocaine hydrochloride is administered in concentrations ranging from 1% to 5%, approximately 37 to 185 mM.[35] Plasma levels achieved after epidural or intravenous anaesthesia are much lower, ranging from 3.5 to 17.3 μM.[36,37] However, the concentrations required for lidocaine formulations to develop cytotoxic and antiproliferative effects can be achieved in some tissues, such as the oral ones, by local injection.[38] Meanwhile, the prolonged exposure time required for lidocaine to induce cytotoxic and antiproliferative effects on SCC9 and SCC25 cells may be a limiting factor.

Lidocaine in lower concentration than HP-β-CD-lido was able to reduce cell proliferation. This difference may be related to a loss in the available drug. Probably, part of the lidocaine molecules remained bound to HP-β-CD and did not interact with the cells. Moreover, the complexation of LA with HP-β-CD evokes a delay on its release and also changes the drug permeation across the membranes.[19,21] However, in the highest concentration (4000 μM), the complexation with HP-β-CD potentiated lidocaine toxicity on SCC9 and SCC25 cells. The increase in the cytotoxic effect in cancer cells by other drugs complexed in HP-β-CD has been described in the literature. Recent studies using norathyriol (polyphenol) and scutellarin (flavonoid) complexed with HP-β-CD showed an enhancement in antiproliferative activity when compared with the free molecule in human colon cancer cells from HCT116, LoVo, SW480 and HT-29 lines.[39,40]

Oxaliplatin, an anticancer agent, had a twofold higher cytotoxicity on HCT116 and MCF-7 cells (breast cancer) when complexed with HP-β-CD.[41] The authors attributed the cytotoxicity potentiation to the increased solubility of the molecules performed by HP-β-CD complexation. Interestingly, treatment with HP-β-CD (4000 μM) alone was also able to cause a small, but significant, reduction in the viability of SCC9 and SCC25 cells. One of the main HP-β-CD features is to have low toxicity.[42] The change observed may be related to a high concentration of the molecule applied to an *in vitro* model.

Conclusion

In conclusion, lidocaine was successfully complexed in HP-β-CD, the local anaesthetic showed cytotoxic effects on human tongue squamous carcinoma cells (SCC9 and SCC25), which were potentiated by HP-β-CD. The complexation into HP-β-CD could be an interesting therapeutic adjuvant; however, the *in vitro* results should be confirmed in the *in vivo* assays.

Declarations

Conflict of interest

The Authors declare that they have no conflicts of interest to disclose.

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References

1. Chitapanarux I *et al.* Oral cavity cancers at a young age: analysis of patient, tumor and treatment characteristics in Chiang Mai University Hospital. *Oral Oncol* 2006; 42: 83–88.
2. Scully C, Felix DH. Oral medicine – update for the dental practitioner oral cancer. *Br Dent J* 2006; 200: 13–17.
3. Santos-Silva AR *et al.* Tongue squamous cell carcinoma in young nonsmoking and nondrinking patients: 3 clinical cases of orthodontic interest. *Am J Orthod Dentofacial Orthop* 2014; 145: 103–107.
4. Bello IO *et al.* Prognostic evaluation of oral tongue cancer: means, markers and perspectives (I). *Oral Oncol* 2010; 46: 630–635.
5. Yuasa-Nakagawa K *et al.* Cervical lymph node metastasis from early-stage squamous cell carcinoma of the oral tongue. *Acta Otolaryngol* 2013; 133: 544–551.
6. Warnakulasuriya S. Living with oral cancer: epidemiology with particular reference to prevalence and life-style changes that influence survival. *Oral Oncol* 2010; 46: 407–410.
7. Piegeler T *et al.* Antimetastatic potential of amide-linked local anesthetics: inhibition of lung adenocarcinoma cell migration and inflammatory Src signaling independent of sodium channel blockade. *Anesthesiology* 2012; 117: 548–559.
8. Ferreira LE *et al.* The effect of two drug delivery systems in ropivacaine cytotoxicity and cytokine release by human keratinocytes and fibroblasts. *J Pharm Pharmacol* 2017; 69: 161–171.
9. Kobayashi K *et al.* Cytotoxicity and type of cell death induced by local anesthetics in human oral normal and tumor cells. *Anticancer Res* 2012; 32: 2925–2933.
10. Wada H *et al.* Combined spinal and general anesthesia attenuates liver metastasis by preserving TH1/TH2 cytokine balance. *Anesthesiology* 2007; 106: 499–506.
11. Mammoto T *et al.* Infiltration anesthetic lidocaine inhibits cancer cell invasion by modulating ectodomain shedding of heparin-binding epidermal growth factor like growth factor (HB-EGF). *J Cell Physiol* 2002; 192: 351–358.
12. Sakaguchi M *et al.* The antiproliferative effect of lidocaine on human tongue cancer cells with inhibition of the activity of epidermal growth factor receptor. *Anesth Analg* 2006; 102: 1103–1107.
13. Grant AS. The holy grail: long-acting local anesthetics and liposomes. *Best Pract Res Clin Anaesthesiol* 2002; 16: 345–352.
14. de Paula E *et al.* Drug delivery systems for local anesthetics. *Recent Pat Drug Deliv Formul* 2010; 4: 23–34.
15. Shikanov A *et al.* Long acting local anesthetic-polymer formulation to prolong the effect of analgesia. *J Controlled Release* 2007; 117: 97–103.
16. Loftsson T, Duchêne D. Cyclodextrins and their pharmaceutical applications. *Int J Pharm* 2007; 329: 1–11.
17. Duchêne D, Wouessidjewe D. The current state of β-cyclodextrin in pharmaceutics. *Acta Pharm Technol* 1990; 36: 1–6.
18. Mura P. Analytical techniques for characterization of cyclodextrin complexes in aqueous solution: a review. *J Pharm Biomed Anal* 2014; 101: 238–250.
19. de Araujo DR *et al.* Development and pharmacological evaluation of ropivacaine-2-hydroxypropyl-beta-cyclodextrin inclusion complex. *Eur J Pharm Sci* 2008; 33: 60–71.
20. Cereda CM *et al.* Local neurotoxicity and myotoxicity evaluation of cyclodextrin complexes of bupivacaine and ropivacaine. *Anesth Analg* 2012; 115: 1234–1241.
21. Moraes C *et al.* Characterization of lidocaine: hydroxypropyl-β-cyclodextrin inclusion complex. *J Incl Phenom Macrocycl Chem* 2007; 57: 313–316.
22. Wei Y *et al.* Masking the bitter taste of injectable lidocaine HCl formulation for dental procedures. *AAPS PharmSciTech* 2015; 16: 455–465.
23. Dollo G *et al.* Inclusion complexation of amide-typed local anesthetics with β-cyclodextrin and its derivatives. III. Biopharmaceutics of bupivacaine-SBE7-β-CD complex following percutaneous sciatic nerve administration in rabbits. *Int J Pharm* 1998; 164: 11–19.
24. Loftsson T, Masson M. Cyclodextrins in topical drug formulations: theory and practice. *Int J Pharm* 2001; 225: 15–30.
25. Höhne G *et al.* *Differential scanning calorimetry – an introduction for practitioners*. Berlin: Springer Verlag, 1996.
26. Silva MS *et al.* Paraquat-loaded alginate/chitosan nanoparticles: preparation, characterization and soil sorption studies. *J Hazard Mater* 2011; 190: 366–374.
27. Pascoal AC *et al.* Antiproliferative activity and induction of apoptosis in PC-3 cells by the chalcone cardamonin from *Campomanesia adamantium* (Myrtaceae) in a bioactivity-guided study. *Molecules* 2014; 19: 1843–1855.
28. Wang WT *et al.* Terfenadine induces anti-proliferative and apoptotic activities in human hormone-refractory prostate cancer through histamine receptor-independent Mcl-1 cleavage and Bak up-regulation. *Naunyn Schmiedebergs Arch Pharmacol* 2014; 387: 33–45.
29. George S, Vasudevan D. Studies on the preparation, characterization, and solubility of 2-HP-β-cyclodextrin-meclizine HCl inclusion complexes. *J Young Pharm* 2012; 4: 220–227.
30. Bar-Yosef S *et al.* Attenuation of the tumor-promoting effect of surgery by spinal blockade in rats. *Anesthesiology* 2001; 94: 1066–1073.
31. Chang YC *et al.* Local anesthetics induce apoptosis in human breast tumor cells. *Anesth Analg* 2014; 118: 116–124.
32. Perez-Castro R *et al.* Cytotoxicity of local anesthetics in human neuronal cells. *Anesth Analg* 2009; 108: 997–1007.
33. Keck M *et al.* Local anesthetics have a major impact on viability of preadipocytes and their differentiation into adipocytes. *Plast Reconstr Surg* 2010; 126: 1500–1505.
34. Jacobs TF *et al.* The effect of Lidocaine on the viability of cultivated mature human cartilage cells: an

in vitro study. *Knee Surg Sports Traumatol Arthrosc* 2011; 19: 1206–1213.

35. Johnson ME *et al.* Mitochondrial injury and caspase activation by the local anesthetic lidocaine. *Anesthesiology* 2004; 101: 1184–1194.
36. Shono A *et al.* Comparison of 1% and 2% lidocaine epidural anaesthesia combined with sevoflurane general anaesthesia utilizing a constant bispectral index. *Br J Anaesth* 2003; 91: 825–829.
37. Kahokehr A *et al.* Systemic levels of local anaesthetic after intraperitoneal application – a systematic review. *Anaesth Intensive Care* 2010; 38: 623–638.
38. Bundscherer AC *et al.* Effects of lidocaine on HT-29 and SW480 colon cancer cells *in vitro*. *Anticancer Res* 2017; 37: 1941–1945.
39. Wang F *et al.* Host-guest inclusion system of scutellarein with 2-hydroxypropyl-beta-cyclodextrin: preparation, characterization, and anticancer activity. *J Biomater Sci Polym Ed* 2014; 25: 594–607.
40. Han B *et al.* Host-guest inclusion system of norathyriol with β-cyclodextrin and its derivatives: preparation, characterization, and anticancer activity. *J Biosci Bioeng* 2014; 117: 775–779.
41. Zhang D *et al.* Preparation, characterisation and antitumour activity of β-, γ- and HP-β-cyclodextrin inclusion complexes of oxaliplatin. *Spectrochim Acta A Mol Biomol Spectrosc* 2016; 152: 501–508.
42. Eida EEM *et al.* Characterization of the inclusion complex of zerumbone with hydroxypropyl-β-cyclodextrin. *Carbohyd Polym* 2011; 83: 1707–1714.

3 CONCLUSÃO

Como conclusão, pode-se afirmar que ambas as formulações estudadas lidocaína (Lido) e lidocaína com 2-hidroxipropil-β-ciclodextrina (Lido-HP-β-CD) afetaram a viabilidade e a taxa de proliferação das células SCC9 e SCC25. A complexação com 2-hidroxipropil-β-ciclodextrina potencializou os efeitos citotóxicos in vitro da lidocaína sobre SCC9 e SCC25. Destarte, embora formulação de doxorrubicina (controle positivo) tenha sido a mais potente, Lido e Lido-HP-β-CD poderiam promover benefícios terapêuticos ao tratamento do carcinoma de células escamosas, conforme observado in vitro. Entretanto, estes resultados precisam ser confirmados em modelos in vivo e, posteriormente, em estudos clínicos.

REFERÊNCIAS*

Andrade ED. Terapêutica medicamentosa em Odontologia. 3.ed. São Paulo: Artes Médicas, 2014.

Andrews E, Seaman WT, Webster-Cyriaque J. Oropharyngeal carcinoma in non-smokers and non-drinkers: a role for HPV. Oral Oncol. 2009 Jun;45(6):486-91. doi: 10.1016/j.oraloncology.2008.07.008.

Bundscherer AC, Malsy M, Bitzinger DI, Wiese CH, Gruber MA, Graf BM. Effects of lidocaine on HT-29 and SW480 colon cancer cells in vitro. Anticancer Res. 2017 Apr;37(4):1941-1945.

Brener S, Jeunon FA, Barbosa AF, Grandinetti HAM, Carcinoma de células escamosas bucal: uma revisão de literatura entre o perfil do paciente, estadiamento clínico e tratamento proposto. Revista Brasileira de Cancerologia 2007; 53(1): 63-69.

Chamaraux-Tran TN, Piegeler T. The Amide Local Anesthetic Lidocaine in Cancer Surgery-Potential Antimetastatic Effects and Preservation of Immune Cell Function? A Narrative Review. Front Med (Lausanne). 2017 Dec 20;4:235. doi: 10.3389/fmed.2017.00235. eCollection 2017.

de Araujo DR, Tsuneda SS, Cereda CM, Del G F Carvalho F, Preté PS, Fernandes SA, Yokaichiya F, Franco MK, Mazzaro I, Fraceto LF, de F A Braga A, de Paula E. Development and pharmacological evaluation of ropivacaine-2-hydroxypropyl-beta-cyclodextrin inclusion complex. Eur J Pharm Sci. 2008 Jan;33(1):60-71.

de Paula E, Cereda CM, Fraceto LF, de Araújo DR, Franz-Montan M, Tofoli GR, Ranali J, Volpato MC, Groppo FC. Micro and nanosystems for delivering local anesthetics. Expert Opin Drug Deliv. 2012 Dec;9(12):1505-24. doi: 10.1517/17425247.2012.738664.

de Paula E, Cereda CM, Tofoli GR, Franz-Montan M, Fraceto LF, de Araújo DR.Drug delivery systems for local anesthetics. Recent Pat Drug Deliv Formul. 2010 Jan;4(1):23-34.

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Ding Z, Xiao T, Huang J, Yuan Y, Ye Q, Xuan M, Xie H, Wang X. Elective Neck Dissection Versus Observation in Squamous Cell Carcinoma of Oral Cavity With Clinically N0 Neck: A Systematic Review and Meta-Analysis of Prospective Studies. J Oral Maxillofac Surg. 2018 Aug 22. pii: S0278-2391(18)30971-6. doi: 10.1016/j.joms.2018.08.007.

Dollo G, Thompson DO, Le Corre P, Chevanne, F, Le Verge R. Inclusion complexation of amide-typed local anesthetics with β-cyclodextrin and its derivatives. III. Biopharmaceutics of bupivacaine-SBE7-β-CD complex following percutaneous sciatic nerve administration in rabbits. Int J Pharm 1998; 164: 11-19.

Eida, EEM, Abdula, AB, Sulimand, FEO, Sukarie, MA, Rasedeef, A, Fataha, SS. Characterization of the inclusion complex of zerumbone with hydroxypropyl-β-cyclodextrin. Carbohydrate Polymers. 2011; 83(4): 1707-1714.

Han B, Yang B, Yang X, Zhao Y, Liao X, Gao C, Wang F, Jiang R. Host-guest inclusion system of norathyriol with β-cyclodextrin and its derivatives: preparation, characterization, and anticancer activity. J Biosci Bioeng. 2014 Jun;117(6):775-9. doi: 10.1016/j.jbiosc.2013.12.001.

Hoornstra D, Vesterlin J, Pärnänen P, Al-Samadi A, Zlotogorski-Hurvitz A, Vered M, Salo T. Fermented lingonberry juice inhibits oral tongue squamous cell carcinoma invasion in vitro similarly to curcumin. In Vivo. 2018 Sep-Oct;32(5):1089-1095. doi: 10.21873/invivo.11350.

INSTITUTO NACIONAL DO CÂNCER (INCA). Câncer da cavidade oral. 2018. [acesso em 2018 Set 25]. Disponível em: http://www.inca.gov.br/estimativa/2018/sintese-de-resultados-comentarios.asp.

Iype EM, Sebastian P, Mathew A, et al: The role of selective neck dissection (I-III) in the treatment of node negative (N0) neck in oral cancer. Oral Oncol. 2008 Dec;44(12):1134-8. doi: 10.1016/j.oraloncology.2008.02.017.

Kobayashi K, Ohno S, Uchida S, Amano O, Sakagami H, Nagasaka H. Cytotoxicity and type of cell death induced by local anesthetics in human oral normal and tumor cells. Anticancer Res. 2012 Jul;32(7):2925-33.

Le Campion ACOV, Ribeiro CMB, Luiz RR, da Silva Júnior FF, Barros HCS, Dos Santos KCB et al. Low Survival Rates of Oral and Oropharyngeal Squamous Cell Carcinoma. Int J Dent. 2017;2017:5815493. doi: 10.1155/2017/5815493.

Liu M, Cao W, Sun Y, He Z. Preparation, characterization and in vivo evaluation of formulation of repaglinide with hydroxypropyl-β-cyclodextrin. Int J Pharm. 2014 Dec 30;477(1-2):159-66. doi: 10.1016/j.ijpharm.2014.10.038.

Liu J, Qiu L, Gao J, Jin Y. Preparation, characterization and in vivo evaluation of formulation of baicalein with hydroxypropyl-beta-cyclodextrin. Int J Pharm. 2006 Apr 7;312(1-2):137-43.

Ma SX, Chen W, Yang XD, Zhang N, Wang SJ, Liu L, Yang LJ. Alpinetin/hydroxypropyl-β-cyclodextrin host-guest system: preparation, characterization, inclusion mode, solubilization and stability. J Pharm Biomed Anal. 2012 Aug-Sep;67-68:193-200. doi: 10.1016/j.jpba.2012.04.038.

Mammoto T, Higashiyama S, Mukai M, Mammoto A, Ayaki M, Mashimo T, Hayashi Y, Kishi Y, Nakamura H, Akedo H. Infiltration anesthetic lidocaine inhibits cancer cell invasion by modulating ectodomain shedding of heparin-binding epidermal growth factor-like growth factor (HB-EGF). J Cell Physiol. 2002 Sep;192(3):351-8.

Moraes C, Abrami P, Araujo D, Braga A, Issa M, Ferraz H, Paula E, Fraceto LF. Characterization of lidocaine:hydroxypropyl-β-cyclodextrin inclusion complex. Journal of Inclusion Phenomena and Macrocyclic Chemistry. 2007; 57(4): 313-316.

Mura P. Analytical techniques for characterization of cyclodextrin complexes in aqueous solution: a review. J Pharm Biomed Anal. 2014 Dec;101:238-50. doi: 10.1016/j.jpba.2014.02.022.

Nóbrega TD, Queiroz SI, Santos EM, Costa AL, Pereira-Pinto L, de Souza LB. Clinicopathological evaluation and survival of patients with squamous cell carcinoma of the tongue. Med Oral Patol Oral Cir Bucal. 2018 Sep 1;23(5):e579-e587. doi: 10.4317/medoral.22421.

Paczkowska M, Mizera M, Lewandowska K, Kozak M, Miklaszewski A, Cielecka-Piontek J. Effects of inclusion of cetirizine hydrochloride in β-cyclodextrin. J Incl Phenom Macrocycl Chem. 2018;91(3):149-159. doi: 10.1007/s10847-018-0808-y.

Perez-Castro R, Patel S, Garavito-Aguilar ZV, Rosenberg A, Recio-Pinto E, Zhang J, Blanck TJ, Xu F. Cytotoxicity of local anesthetics in human neuronal cells. Anesth Analg. 2009 Mar;108(3):997-1007. doi: 10.1213/ane.0b013e31819385e1.

Sakaguchi M, Kuroda Y, Hirose M. The antiproliferative effect of lidocaine on human tongue cancer cells with inhibition of the activity of epidermal growth factor receptor. Anesth Analg. 2006 Apr;102(4):1103-7.

Wada H et al. Combined spinal and general anesthesia attenuates liver metastasis by preserving TH1/TH2 cytokine balance. Anesthesiology. 2007 Mar;106(3):499-506.

Wang F, Yang B, Zhao Y, Liao X, Gao C, Jiang R, Han B, Yang J, Liu M, Zhou R. Host-guest inclusion system of scutellarein with 2-hydroxypropyl-beta-cyclodextrin: preparation, characterization, and anticancer activity. J Biomater Sci Polym Ed. 2014;25(6):594-607. doi: 10.1080/09205063.2014.884875.

Warnakulasuriya S: Global epidemiology of oral and oropharyngeal cancer. Oral Oncol. 2009 Apr-May;45(4-5):309-16. doi: 10.1016/j.oraloncology.2008.06.002.

Yang X, Zhao L, Li M, Yan L, Zhang S, Mi Z, Ren L, Xu J. Lidocaine enhances the effects of chemotherapeutic drugs against bladder cancer. Sci Rep. 2018 Jan 12;8(1):598. doi: 10.1038/s41598-017-19026-x.

Zhang D, Zhang J, Jiang K, Li K, Cong Y, Pu S, Jin Y, Lin J. Preparation, characterisation and antitumour activity of β-, γ- and HP-β-cyclodextrin inclusion complexes of oxaliplatin. Spectrochim Acta A Mol Biomol Spectrosc. 2016 Jan 5;152:501-8. doi: 10.1016/j.saa.2015.07.088.

Zhang L, Hu R, Cheng Y, Wu X, Xi S, Sun Y, Jiang H. Lidocaine inhibits the proliferation of lung cancer by regulating the expression of GOLT1A. Cell Prolif. 2017 Oct;50(5). doi: 10.1111/cpr.12364.

ANEXOS

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