Journal of the Formosan Medical Association (2014) 113, 591–599

Available online at www.sciencedirect.com

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journal homepage: www.jfma-online.com

ORIGINAL ARTICLE

Topical methotrexate pretreatment enhances the therapeutic effect of topical 5-aminolevulinic acid-mediated photodynamic therapy on hamster buccal pouch precancers

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Received 7 January 2014; received in revised form 24 February 2014; accepted 19 March 2014

KEYWORDS
5-aminolevulinic acid;
hamster buccal pouch precancer;
methotrexate;
oral precancerous lesions;
topical photodynamic therapy

Background/Purpose: Topical 5-aminolevulinic acid-mediated photodynamic therapy (ALA-PDT) is effective for treatment of human oral precancerous lesions. This animal study aimed to assess whether topical methotrexate (MTX) pretreatment could enhance the therapeutic effect of topical ALA-PDT on hamster buccal pouch precancerous lesions.
Methods: Twenty hamster buccal pouch precancerous lesions were treated with either topical ALA-PDT with topical MTX pretreatment (topical MTX-ALA-PDT group, $n = 10$) or topical ALA-PDT alone (topical ALA-PDT group, $n = 10$). The intracellular protoporphyrin IX (PpIX) level in another 12 precancerous lesions ($n = 6$ for either the topical MTX-ALA or topical ALA group) was monitored by fluorescence spectroscopy.
Results: The intracellular PpIX reached its peak level in precancerous lesions 6.5 hours and 2.5 hours after topical ALA application for the topical MTX-ALA group (5.63-fold higher in the lesion than in the normal mucosa) and topical ALA group (2.42-fold higher in the lesion than in the normal mucosa), respectively. The complete response rate of precancerous lesions was 80% for the topical MTX-ALA-PDT group and 70% for the topical ALA-PDT group. In addition, the topical MTX-ALA-PDT group required a significantly lower mean treatment number

Conflicts of interest: The authors have no conflicts of interest relevant to this article.

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http://dx.doi.org/10.1016/j.jfma.2014.03.002

(2.1 ± 0.6) to achieve complete response than the topical ALA-PDT group (4.4 ± 1.3, $p < 0.001$)). Moreover, the topical MTX-ALA-PDT group had a lower recurrence rate (12.5%) than the topical ALA-PDT group (28.6%).
Conclusion: We conclude that topical MTX-pretreatment can increase intracellular PpIX production in hamster buccal pouch precancerous lesions and significantly improves the outcomes of the precancerous lesions treated with topical ALA-PDT.

Introduction

Oral leukoplakia, erythroleukoplakia, and verrucous hyperplasia are three common oral precancerous lesions. The malignant transformation rates are reported to be 1—7% for homogenous thick oral leukoplakia, 4—15% for granular or verruciform oral leukoplakia, and 18—47% (28% in average) for oral erythroleukoplakia.[1] A retrospective clinical study showed a malignant transformation rate of 3.1% for 324 oral verrucous hyperplasia lesions arising from Taiwanese patients.[2] Our previous study also demonstrated a 5-year malignant transformation rate of 3% for 30 plaque-typed and of 17% for 30 mass-typed oral verrucous hyperplasia lesions.[3] In Taiwan, advanced oral cancer patients are prone to having a poor prognosis.[4,5] The high malignant transformation rates of oral precancers and poor prognosis for patients with advanced oral cancers highlight the importance of early detection and treatment of oral precancers and cancers.

Traditional treatment for oral precancers is total surgical excision that always leads to scar formation for a large precancerous lesion.[6] Photodynamic therapy (PDT) is another effective treatment option for human oral precancerous and cancerous lesions, because it is noninvasive, is well tolerated by patients, can be used repeatedly without cumulative side effects, and results in little scar formation.[7] 5-Aminolevulinic acid (ALA) itself is not a photosensitizer but serves as the biological precursor of the photosensitizer, protoporphyrin IX (PpIX), in the heme biosynthesis pathway. ALA is superior to other photosensitizers because it can be rapidly cleared from the tissues and the body within 48 hours and patients after ALA-mediated PDT (ALA-PDT) treatment have no problem of prolonged skin photosensitivity.[8,9]

PDT involves mainly two individually nontoxic components, light and the photosensitizer. When a photosensitizer in tissues is activated by a light of a specific wavelength, it transfers energy from the light to molecular oxygen, resulting in the generation of reactive oxygen species (ROS).[7] There are three main mechanisms by which PDT mediates tumor destruction. Firstly, the ROS can kill tumor cells directly. Secondly, PDT can damage the tumor-associated vasculature, leading to thrombus formation and subsequent tumor infarction. Thirdly, PDT can also activate an immune response against tumor cells.[7]

Previous studies have shown that ALA-PDT is effective for treatment of both oral cancer and precancers.[10—23] However, four to six topical ALA-PDT treatments are needed to achieve a complete regression of the relatively large oral precancerous lesions.[18—23] Previous studies found that pretreatment of prostate cancer cells and skin keratinocytes or carcinoma cells with a low-dose methotrexate (MTX) can have a 2—4-fold upregulation of expression of coproporphyrinogen oxidase (CPOX) proteins, resulting in a 2—4-fold increase in intracellular PpIX production and subsequent killing of these epithelial or cancer cells after exposure to the specific light.[24—29] Our previous studies showed that both topical ALA-PDT and topical photosan-mediated PDT are very effective for the treatment of hamster buccal pouch precancerous lesions.[30,31] Our recent study found that the SCC4 cells pretreated with 0.001 mg/L MTX for 3 days showed a significant and 1.65-fold increase in CPOX expression compared with the control SCC4 cells without MTX pretreatment.[32] Therefore, we hypothesize that when hamster buccal pouch precancerous lesions are pretreated with a low and nontoxic topical dose of MTX and then treated with 100-J topical ALA-PDT (fluence rate, 200 mW/cm^2; light dose, 100 J/cm^2), the PDT efficacy can be augmented and the PDT treatment number to achieve a complete regression of the lesion can be reduced. In this study, 20 hamster buccal pouch precancerous lesions were treated with either the topical ALA-PDT with topical MTX pretreatment for 3 days (topical MTX-ALA-PDT group, $n = 10$) or topical ALA-PDT without MTX-pretreatment (topical ALA-PDT group, $n = 10$). We tried to assess whether topical MTX-pretreatment could increase intracellular PpIX production in hamster buccal pouch precancerous lesions and significantly improved the outcomes of precancerous lesions treated with topical ALA-PDT.

Materials and methods

Establishment of hamster buccal pouch carcinogenesis model

A total of 38 adult male Syrian golden hamsters (10-week-old) purchased from the National Laboratory Animal Center, Taipei, Taiwan were used in this study. This study was approved (Permit Number: 9915) and carried out in strict accordance with the recommendations in the Guide for the Care and Use of the Institutional Animal Care and Use Committee of Chung Yuan Christian University, Chungli, Taiwan. 7,12-Dimethylbenz(a)anthracene (DMBA; Sigma-Aldrich, St. Louis, MO, USA) solution (0.5%, w/w) was prepared by adding 0.5 g of DMBA in 99.5 g mineral oil. DMBA solution was applied to the left buccal pouches three times/week for 9—10 weeks to induce the formation of oral