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Enhancement of the photodynamic effects on human oral squamous cell carcinoma cell lines by treatment with calcipotriol

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KEYWORDS
Photodynamic therapy;
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Summary

Background: 5-Aminolaevulinic acid (ALA)-based photodynamic therapy (PDT) for patients with skin and oral diseases is a highly sophisticated procedure, but the incidence of disease recurrence after treatment with ALA-based PDT is somewhat alarming. Calcipotriol, an analogue of 1,25-dihydroxyvitamin D3 (1,25(OH)2D3), has been reported to regulate the proliferation and differentiation of keratinocytes.
Objective: In order to obtain even greater efficacy of ALA-based PDT, we investigated the synergistic effects of calcipotriol as an adjunct to ALA-based PDT for human oral squamous cell carcinoma (SCC) cell lines.

Abbreviations: 1,25(OH)2D3, 1,25-dihydroxyvitamin D3; ALA, 5-aminolaevulinic acid; COX-2, cyclooxygenase-2; PDT, photodynamic therapy; PpIX, protoporpophyrin IX; SCC, squamous cell carcinoma

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Methods: Intracellular protoporphyrin IX (PpIX) converted from exogenous ALA in SCC cell lines treated with/without calcipotriol was measured by a fluorescencemeter. Then, the *in vitro* effects of calcipotriol, the cyclooxygenase (COX)-2 selective inhibitor (nimesulide), ALA-based PDT and their combination on two SCC cell lines, HSC-2 (a *COX-2* high expresser) and HSC-4 (a *COX-2* non-expresser), were determined by MTT assay and double-staining for annexin V and propidium iodide.
Results: The concentration of intracellular PpIX was increased in four of the eight SCC cell lines (50%) treated with calcipotriol. The greatest alteration of intracellular PpIX was found in HSC-4 (1.9-fold). The combination of calcipotriol and ALA-based PDT remarkably inhibited cellular proliferation and induced cellular death of both HSC-2 and HSC-4. Whereas, this morphological damage was more serious in HSC-4 than in HSC-2. Furthermore, these effects were almost equivalent to the synergistic effect of the combination of nimesulide and ALA-based PDT on HSC-2.
Conclusions: The present study suggests that treatment with calcipotriol enhances the photodynamic effects on SCC via the accumulation of exogenous ALA-dependent PpIX.

1. Introduction

5-Aminolaevulinic acid (ALA)-based photodynamic therapy (PDT) is widely applied to patients with cancer and pre-cancerous lesions. The photosensitizer protoporphyrin IX (PpIX), converted *in situ* from exogenous ALA, is essential for the photodynamic effects as the mechanism mediated by PpIX and visible light leads to the formation of intracellular singlet oxygen that plays a central role in photodynamic cytotoxicity [1—3]. Recent studies have revealed the efficacy of ALA-based PDT for squamous cell carcinoma (SCC) and SCC *in situ*, Bowen's diseases and actinic keratoses [1,2,4]. In spite of the advantages of ALA-based PDT, such as easy accessibility, selective cell killing and having no noticeable adverse effects, we still have to overcome a high incidence of disease recurrence experienced by the patients after undergoing the treatment [5,6]. We, as well as others, reported that ALA-based PDT has the potential of inducing an apoptotic process specifically limited to the abnormal/neoplastic cells with little effects on the normal cells [4]. Moreover, our previous study demonstrated that cyclooxygenase (COX)-2 could be one of the molecular targets of a treatment approach in conjunction with ALA-based PDT for various disorders in the skin and oral cavity [7].

1,25-Dihydroxyvitamin D3 (1,25(OH)2D3) binds to the nuclear Vitamin D receptor, and acts as a transcription factor, and then induces anti-proliferation, differentiation and apoptosis of various normal and cancer cells [8—19]. 1,25(OH)2D3 has been successfully applied for several hyperproliferative skin diseases, although its potent effect on calcium metabolism has limited most clinical applications [12,20]. Calcipotriol (MC903), a synthetic analogue of 1,25(OH)2D3, is much less potent in terms of calcium metabolism, but retains similar effects on cell proliferation and differentiation in various cell types [10,12,15,18—21]. Calcipotriol has already been topically applied for patients with psoriasis and is one of the promising methods for the treatment of skin diseases together with a combination of other therapies. Recently, it was also reported that 1,25(OH)2D3 and its analogues increased ALA-induced PpIX and enhanced the photodynamic effect on LNCaP prostate cancer cells [22].

In order to obtain an even greater efficacy of ALA-based PDT, we investigated whether calcipotriol could be applied to the treatment approach in conjunction with ALA-based PDT for skin and oral lesions. In the present study, firstly, intracellular PpIX converted from exogenous ALA in eight human oral SCC cell lines treated with/without calcipotriol was examined by a fluorescencemeter. We then tested the synergistic effects *in vitro* with the combination of calcipotriol, a COX-2 selective inhibitor (nimesulide), ALA-based PDT and their combination on two SCC cell lines, HSC-2 (a *COX-2* high expresser) and HSC-4 (a *COX-2* non-expresser) by MTT assay and double-staining for annexin V and propidium iodide (PI).

2. Materials and methods

2.1. Chemicals

ALA was obtained from Sigma (St. Louis, MO, U.S.A.). Calcipotriol was kindly donated by Dr. Lise Binderup (Leo Pharmaceutical Products, Ballerup, Denmark).