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Research paper

In vitro phototoxicity of 5-aminolevulinic acid and its methyl ester and the influence of barrier properties on their release from a bioadhesive patch

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Abstract

Topical administration of excess exogenous 5-aminolevulinic acid (ALA) leads to selective accumulation of the potent photosensitiser protoporphyrin IX (PpIX) in neoplastic cells, which can then be destroyed by irradiation with visible light. Due to its hydrophilicity, ALA penetrates deep lesions, such as nodular basal cell carcinomas (BCCs) poorly. As a result, more lipophilic esters of ALA have been employed to improve tissue penetration. In this study, the in vitro release of ALA and M-ALA from proprietary creams and novel patch-based systems across normal *stratum corneum* and a model membrane designed to mimic the abnormal *stratum corneum* overlying neoplastic skin lesions were investigated. Receiver compartment drug concentrations were compared with the concentrations of each drug producing high levels of PpIX production and subsequent light-induced kill in a model neoplastic cell line (LOX). LOX cells were found to be quite resistant to ALA- and M-ALA-induced phototoxicity. However, drug concentrations achieved in receiver compartments were comparable to those required to induce high levels of cell death upon irradiation in cell lines reported in the literature. Patches released significantly less drug across normal *stratum corneum* and significantly more across the model membrane. This is of major significance since the selectivity of PDT for neoplastic lesions will be further enhanced by the delivery system. ALA/M-ALA will only be delivered in significant amounts to the abnormal tissue. PpIX will only then accumulate in the neoplastic cells and the normal surrounding tissue will be unharmed upon irradiation.

Keywords: Photodynamic therapy; 5-Aminolevulinic acid; *Stratum corneum*; Patches; Drug delivery; Bioadhesion

1. Introduction

Photodynamic therapy (PDT) based on topical application of 5-aminolevulinic acid (ALA) has been shown to achieve high clearance rates when used in the treatment of superficial skin lesions, such as basal cell carcinoma, Bowen's disease and actinic keratosis [1]. ALA, a naturally occurring precursor in the biosynthetic pathway of haem, is now the most commonly used drug in modern dermatological PDT. Topical administration of excess exogenous ALA leads to selective accumulation of the potent photosensitiser protoporphyrin IX (PpIX) in neoplastic cells which can then be destroyed by irradiation with visible light. Excellent tissue preservation and lack of scarring are noted advantages over conventional surgical treatment options for such lesions. However, deep lesions, such as nodular basal cell carcinomas, or those with overlying keratinous debris are reported as being resistant to ALA PDT [1,2]. Such treatment failures have been attributed to the water solubility of the drug preventing its deep penetration into tissue [1]. Consequently, more lipophilic ALA derivatives have been produced with the aim of improving drug penetration and subsequent treatment success rates. The

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most commonly investigated ALA derivative has been its methyl ester (M-ALA). Improved clearance rates of nodular basal cell carcinomas have been reported after topical application of M-ALA [3].

ALA and M-ALA are typically delivered to surface lesions using topically applied creams which are covered with occlusive dressings to aid retention at the site and enhance drug absorption [4]. However, great variability in the amount of such creams applied per unit area has been reported [1,5–7]. Application of occlusive dressings over the applied creams leads to smudging and spreading of the cream away from the site of application in an irreproducible fashion, adding further uncertainty to the technique. Consequently, comparison of the results of different studies is difficult. Clearly there is a need for a unit dosage form for use in PDT based on the topical application of ALA or its derivatives, such as M-ALA. Use of such a system would eliminate the inter-clinician variability seen at present and would allow accurate critical comparisons of different studies to be made. An ideal dosage form would be self-adhesive and backed with an occlusive material, thereby negating the need for a covering dressing and simplifying treatment.

This paper describes the design of a bioadhesive patch intended as a topical delivery system for ALA or M-ALA. Drug release from the system across a model membrane and excised *stratum corneum* is evaluated and compared in each case with that from the relevant proprietary cream. Treatment success depends on attainment of a concentration of ALA or M-ALA sufficient to yield photosensitising concentrations of PpIX in viable neoplastic tissue. Therefore, the concentrations of ALA or M-ALA produced on the receptor side of a model membrane and excised *stratum corneum* are compared with the concentrations shown to be phototoxic to a model cell line derived from a skin neoplasm.

2. Materials and methods

2.1. Chemicals

Gantrez® AN-139, a copolymer of methylvinylether and maleic anhydride (PMVE/MA), was provided by ISP Co. Ltd., Guildford, UK. Plastisol® medical grade poly(vinyl chloride) emulsion containing diethylphthalate as plasticiser was provided by BASF Coatings Ltd., Clwyd, UK. 5-Aminolevulinic acid, hydrochloride salt and Porphin® cream were purchased from Crawford Pharmaceuticals, Milton Keynes, UK. Triton® X-100 was purchased from Amersham Biosciences, Bucks, UK. 5-Aminolevulinic acid, methyl ester, hydrochloride salt, trypsin type III and tripropylene glycol methyl ether (Dowanol™ TPM), Dulbecco's modified Eagle's medium (DMEM), RPMI 1640 medium, non-essential amino acids, sodium pyruvate, cell dissociation solution, phosphate buffered saline tablets, ethylenediaminetetraacetic acid (EDTA) and trypsin/EDTA solution, trypan blue solution (0.4%), dimethyl sulphoxide and L-glutamine were purchased from Sigma–Aldrich, Dorset, UK. Foetal calf serum (FCS) was purchased from Gibco Ltd. (Paisley, Scotland). Nu-serum was purchased from BD Biosciences (Bedford, MA). Penicillin/streptomycin solution was supplied by Invitrogen Life Technologies Ltd. (Paisley, Scotland). Metvix® cream was provided by Galderma UK Ltd., Hertfordshire, UK. All other chemicals were of analytical reagent grade.

2.2. Membranes for drug release

Cuprophan® dialysis membrane sheets, molecular weight cut-off 10,000 Da, were obtained from Medicell International, London, UK. Porcine skin is a good model for human skin with regard to hair sparseness, presence of subcutaneous fat, epidermal proliferation, and both the orientation and distribution of blood vessels [8,9]. Stillborn piglets were obtained from a local abattoir and abdominal full-thickness skin was immediately excised. Subcutaneous fat and connective tissue were removed using forceps after immersion in water at 60 °C for 2 min [10,11]. The *stratum corneum* was separated from the epidermis after floating on a solution of trypsin type III (0.1% w/v) and sodium bicarbonate (0.5% w/v) at room temperature as described by Bentley et al. [12]. The enzyme digests the nucleated epidermal tissue, enabling the remnants to be removed by gentle rubbing with cotton wool. The *stratum corneum* sheets were rinsed repeatedly with distilled water, gently pressed between tissue paper, spread on filter paper and subsequently dried by storage in a desiccator over silica gel for a maximum of 2 weeks before use.

2.3. Cell line

Cells of the line LOX, derived from a human amelanotic melanoma, were maintained in a 1:1 mixture of Dulbecco's modified Eagle's medium (DMEM) and RPMI 1640 medium supplemented with 10% FCS, 5% Nu-serum, 2 mM L-glutamine, 0.1 mM non-essential amino acids, 1 mM sodium pyruvate, penicillin (1.0 IU ml^{-1}) and streptomycin (1.0 μg ml^{-1}). Cells were routinely subcultured once weekly and maintained at 37 °C and 5% CO_2 in a moist environment. The cell line was kindly provided by Prof. Øystein Fodstad, Norwegian Radium Hospital, Montebello, Oslo, Norway.

2.4. Patch manufacture

Bioadhesive patches evaluated in this study were prepared by a conventional casting technique [13] using a 20% w/w PMVE/MA and 10% w/w TPM gel. PMVE/MA was added to ice-cooled water (reagent grade 1), stirred vigorously and heated to 95 °C until a clear solution was formed. Upon cooling, the required amount of tripropylene glycol methyl ether (TPM) was added and the casting blend adjusted to a final weight with water. Appropriate amounts of ALA and M-ALA were dissolved