Novel patch-based systems for the localised delivery of ALA-esters

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ABSTRACT

In photodynamic therapy (PDT) a combination of visible light and a sensitising drug causes the destruction of selected cells. Aminolaevulinic acid (ALA) has been widely used in topical PDT for over 15 years. However, ALA does not possess favourable physicochemical properties for skin penetration. Consequently, the clearance rates for difficult to treat lesions, such as nodular basal cell carcinomas are relatively low.

For the first time, equimolar concentrations of ALA, methyl-ALA (m-ALA) and hexyl-ALA (h-ALA) have been incorporated into a bioadhesive patch-based system. *In vitro* penetration studies into excised porcine skin revealed that ALA patches containing relatively high loadings ($226.7 \, \mu mol \, cm^{-2}$) were associated with significantly greater tissue concentrations ($70.7 \, \mu mol \, cm^{-3}$) than patches containing m-ALA ($16.3 \, \mu mol \, cm^{-3}$) or h-ALA ($17.4 \, \mu mol \, cm^{-3}$). ALA was also found to be the most efficient inducer of protoporphyrin (PpIX) fluorescence in mice, *in vivo* (maximum mean fluorescence: ALA = 236.2 a.u., m-ALA = 175.1 a.u., h-ALA = 193.5 a.u.). However, when the lipophilic hexylester was formulated in a pressure sensitive adhesive (PSA) patch, significantly higher PpIX levels were achieved compared to all bioadhesive systems tested. Of major importance, PSA patches containing relatively low h-ALA loadings induced high PpIX levels, which were localised to the application area.

This study has highlighted the importance of rational selection of both the active agent and the delivery system. Bioadhesive preparations containing ALA are ideal for delivery to moist environments; whereas h-ALA-loaded PSA systems may facilitate enhanced delivery to dry areas of skin. In addition, owing to the relatively low loadings of h-ALA required in PSA patches, the costs of clinical PDT may potentially be reduced.

1. Introduction

Photodynamic therapy (PDT) is a clinical treatment whereby cell death can be induced through the interaction of light and photosensitising chemicals [1]. It involves the administration of a photosensitising agent, which when introduced into the body will accumulate in rapidly dividing cells, followed by subsequent irradiation of the target tissue with a measured light dose of appropriate wavelength [2]. This irradiation, matched to the absorption characteristics of the photosensitiser, results in the activation of the drug through a series of electronic excitations, leading to a series of complex cytotoxic reactions, either dependent on or independent of, the generation of reactive oxygen species [3].

To date, no pre-formed photosensitiser has been licensed for topical application to neoplastic skin lesions. Many pre-formed photosensitisers have relatively high molecular weights (>500 Daltons) or an extreme in polarity. Consequently, skin penetration is poor. The most commonly employed agent in topical PDT is 5-aminolaevulinic acid (ALA). ALA is a small molecular weight (167.6 Daltons) precursor of the endogenous photosensitiser, protoporphyrin IX (PpIX). Topical application of ALA leads to an overproduction of PpIX, which can be subsequently irradiated, as described above. Topical ALA-PDT has been shown to be highly effective for the treatment of superficial skin lesions. However, owing to the hydrophilic nature of ALA (octanol: water partition coefficient ($\log P_{ow}$) of -1.5 [4]), complete clearance rates for deeper neoplasias, such as nodular basal cell carcinoma are not as successful.

Recently, several innovative strategies have been employed to improve ALA penetration into skin, including needle-free jet injection [5], microneedles [6], and iontophoresis [7]. However, one of the most widely-studied approaches for enhancing ALA delivery has been the use of more lipophilic prodrugs of ALA [8–10]. Whilst cell culture studies have clearly demonstrated benefits of using ALA prodrugs [10–13], in vitro skin penetration studies and in vivo animal studies have generally failed to replicate these findings [14–16]. Recently, we have highlighted the fact that cell culture experiments have been carried out using equimolar concentrations of ALA and ALA-esters, whereas skin penetration studies have been carried out using formulations containing equal concentrations in terms of% w/w [17]. The first step in the haem biosynthesis pathway is the conversion of two ALA molecules into porphobilinogen. Clearly, due to the greater molecular weight of ALA prodrugs, the number of moles of ALA present in a given mass of prodrug will be less than for the same mass of ALA. Consequently, the molar amount of ALA available for PpIX production is reduced and may negate any benefit derived by using the prodrug. We demonstrated that, at equimolar concentrations, increased levels of the hexylester were found in the upper layers of excised porcine skin compared to ALA and m-ALA. Importantly, the hexylester was also shown to be significantly more efficient at inducing PpIX in vivo. We also highlighted that a cream base became less viscous upon the addition of increasing loadings of h-ALA, an observation also noted elsewhere [14,18,19]. Consequently, when an occlusive dressing was used to retain the formulations in place, the h-ALA cream spreads far beyond the initial site of the application. This is associated with a number of problems. Spreading of the formulation makes it very difficult to ascertain the amount of drug that has actually been applied to a given area. This leads to the possibility of under- or overdosing and makes comparison of different clinical studies very difficult. In addition, spreading of the formulations may lead to photosensitivity outside of intended treatment area.

Previously, it has been shown that patches possess a number of advantages over the conventional cream preparations routinely employed in PDT [20,21]. Importantly, such patches have been shown to localise the delivery of ALA to the area of application and provide a means of standardising dosing. The aim of this study was to incorporate ALA and ALA-esters into novel patch-based systems at equimolar concentrations and to evaluate their delivery in vitro and in vivo.

2. Materials and methods

2.1. Chemicals

5-Aminolaevulinic acid hydrochloride salt (ALA) was supplied by Crawford Pharmaceuticals, Milton Keynes, UK. Methyl 5-aminolaevulinate hydrochloride salt (m-ALA), acetyl acetone, thionyl chloride and hexanol were supplied by Sigma Aldrich, Dorset, UK. Gantrez AN-139, a copolymer of methyl vinyl ether and maleic anhydride (PMVE/MA), was provided by ISP Co. Ltd. (Guildford, UK). Plastisol® medical grade poly(vinyl chloride) emulsion containing diethylphthalate as plasticizer was provided by BASF Coatings Ltd. (Clwyd, UK). Tissue-TEK® tissue embedding fluid was provided by Sakura Finetech Europe B.V., Zoeterwade, The Netherlands. NCS®-II Tissue solubiliser was obtained from Amersham Biosciences Bucks, UK. Ultima Gold® Liquid Scintillation Cocktail and radiolabelled 5-aminolaevulinic acid (ALA) solution, 3.7 MBq ml^{-1} were obtained from PerkinElmer Life Sciences, Beaconsfield, Bucks, UK. Duro-Tak® 387-2054 was provided by National Starch and Chemical Company, Berkely, CA, USA. Sevofluran was obtained

from Abbot, Germany. All other chemicals were of analytical reagent grade.

2.2. Synthesis of hexyl-ALA and radiolabelled esters

ALA hexylester was synthesised by reacting ALA with hexanol, as described previously [22]. Briefly, hexanol (3.5 ml) was cooled to −10 °C in a methanol–ce bath. Thionyl chloride (0.5 ml) was added to the hexanol at such a rate that the temperature did not rise above 10 °C. ALA hydrochloride salt (0.5 g) was added whilst stirring. The solution was heated to 70 °C until the ALA was completely dissolved. Upon cooling to room temperature, diethyl ether (30 ml) was added and the solution stored at −70 °C for 2 h. The resultant precipitate was then filtered under vacuum, and dried. The ester was recrystallised by dissolving it in 1.5 ml of methanol, to which 8.5 ml of diethyl ether was then added. After cooling for 2 h (−70 °C), the ester was filtered off, washed with ether and dried at 40 °C.

Radiolabelled h-ALA was prepared in the same way, with the additional step of adding 100 μl of ^{14}C ALA to the reaction mixture prior to heating. Radiolabelled methyl-ALA (m-ALA) was also synthesised in this way, with hexanol being replaced with methanol.

2.3. Preparation of bioadhesive patch formulations

ALA and ALA-ester containing bioadhesive patches were prepared by modification of methods described previously [20]. Briefly, ALA-containing films were cast from aqueous blends containing 20% w/w poly(methylvinylether/maleic anhydride) (PMVE/MA) and 10% tripropyleneglycol methyl ether (TPM). To the appropriate mass of blend, a suitable amount of ALA was added to give films with constant ALA loadings, 5, 19 and 38 mg cm^{-2}. Clinical PDT is frequently carried out with ALA concentrations ranging from 2% to 20%. In terms of the amount of ALA applied per unit area, this translates to a range of approximately 4–40 mg ALA cm^{-2}. Consequently, three ALA loadings were investigated, based on this range. ALA-ester loaded patches were prepared by dissolving the desired amounts of m-ALA or h-ALA in appropriate amounts of aqueous blend to give films of equivalent molar concentrations to the ALA films described in Table 1. For patches containing ^{14}C, the radiolabelled source was added to the gel with stirring. Sufficient radioactivity was added to give approximately 5.9×10^5 disintegrations per minute (dpm) per cm^2 applied.

Films were prepared by slowly casting the drug-loaded gel into a pre-levelled mould, lined with a release liner to facilitate removal of the film. This was placed in a constant air flow at 25 °C in a fume cupboard. Films were removed from the mould by simply peeling the release liner, with attached film, off the base of the mould. Bilaminar bioadhesive patches were prepared by attaching, with the aid of gentle pressure, the exposed side of the films containing drug, to equivalent areas of PVC backing films, prepared by heating Plastisol® emulsion to 160 °C for 15 min. For protection, the release liner was allowed to remain with its siliconised side attached to what had now become the release surface of the formed patch. Once prepared, all formulations were used within 24 h.

Table 1
Molar equivalent amounts of ALA, m-ALA and h-ALA.

ALA loading (mg cm^{-2})	Molar concentration (μmol cm^{-2})	m-ALA loading (mg cm^{-2})	h-ALA loading (mg cm^{-2})
5	29.8	5.4	7.6
19	113.4	20.5	28.4
38	226.7	41.0	57.1