Hexyl Aminolaevulinate Is a More Effective Topical Photosensitiser Precursor than Methyl Aminolaevulinate and 5-Aminolaevulinic Acids When Applied in Equimolar Doses

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ABSTRACT: Aminolaevulinic acid (ALA) is known to poorly penetrate into thick lesions, such as nodular basal cell carcinomas. Short chain ALA esters, possessing increased lipophilicity relative to their hydrophilic parent, have previously been shown to be highly efficient at inducing protoporphyrin IX (PpIX) production in cell culture, at equimolar concentrations. In contrast, *in vitro* skin permeation and *in vivo* animal studies, which up to now have compared prodrugs on a % w/w basis, have failed to demonstrate such benefits. For the first time, equimolar concentrations of ALA, methyl-ALA (m-ALA) and hexyl-ALA (h-ALA) have been incorporated into an o/w cream preparation. *In vitro* penetration studies into excised porcine skin revealed that increased levels of h-ALA, compared to ALA and m-ALA were found in the upper skin layers, at all drug loadings studied. Topical application of the formulations to nude murine skin *in vivo*, revealed that creams containing h-ALA induced significantly higher levels of peak PpIX fluorescence ($F_{max} = 289.0$) at low concentrations compared to m-ALA ($F_{max} = 159.2$) and ALA ($F_{max} = 191.9$). Importantly, this study indicates that when compared on an equimolar basis, h-ALA has improved skin penetration, leading to enhanced PpIX production compared to the parent drug and m-ALA. J Pharm Sci 99:3486–3498, 2010

Keywords: 5-aminolaevulinic acid; prodrugs; topical; esters; drug delivery; skin; percutaneous

INTRODUCTION

Photodynamic therapy (PDT) is a medical treatment by which a combination of visible light and a sensitising drug causes the destruction of selected cells.[1] A drug without dark toxicity is introduced into the body and accumulates preferentially in neoplastic cells. A measured light dose of appropriate wavelength is then used to irradiate the target tissue.[2] This activates the drug and elicits the toxic reaction in the presence of oxygen.[1] Owing to their relatively high molecular weights, preformed photosensitisers do not penetrate into skin effectively.[3] However, topical PDT can be successfully carried out with 5-aminolaevulinic acid (ALA). ALA is a small molecular weight (167.6 Da) precursor of the endogenous photosensitiser, protoporphyrin IX (PpIX). Topical ALA-PDT has been shown to be highly effective for the treatment of superficial skin lesions. However, clearance rates for deeper neoplasias, such as nodular basal cell carcinoma are not as successful. This is likely to be due to the hydrophilic nature of ALA, octanol/water partition coefficient ($\log P_{ow}$) of -1.5.[4] As a result, topically applied ALA penetrates intact *stratum corneum* poorly.[5,6] Although many skin lesions have disordered epithelial barriers, which allow enhanced ALA penetration, its low lipophilicity prevents it from penetrating significantly into hyperkeratotic or deep nodular lesions.[7]

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One of most commonly employed strategies for enhancing topical and transdermal drug delivery is the use of prodrugs. The term prodrug is usually applied to compounds that are inactive in their parent form, but which, following administration, are converted to the active form by skin enzymes.[8] Numerous ALA prodrugs, of varying lipophilicities, have been synthesised by reaction at either the amino group[9–12] or carboxylic acid group.[9,13,14] To date, most ALA prodrugs have been evaluated using *in vitro* cell culture techniques or measuring ALA-induced PpIX production *in vivo*, following topical application of the prodrug. ALA amides and their esters generally fail to induce large amounts of PpIX *in vitro*[9,11] and *in vivo*.[11]

ALA-induced formation of PpIX depends on the penetration of ALA through the cell membrane. Being a zwitterion with pK_a values of 4 (carboxylic acid group) and 8.9 (amino group), the lipophilicity of ALA is unlikely to change significantly in the physiological pH range. It is expected, therefore, that ALA is unlikely to enter cells by passive diffusion alone. Indeed, in bacterial cells, ALA transport into cells is thought to be mediated by the dipeptide permease.[15] Rud et al.[16] showed that ALA is transported into human adenocarcinoma cells by β-amino acid and γ-aminobutyric acid carriers and is Na^+ and partly Cl^- dependant. The PEPT1 and PEPT2 transporters have also been identified as potential transporter systems for ALA uptake.[17]

The methyl ester of ALA has been shown to be taken up actively by WiDr cells using transporters of nonpolar amino acids.[18] However, longer chain aliphatic ALA-esters are not transported by these carriers and it has been postulated that they may enter cells by either passive diffusion or endocytosis.[19] Once in the cell, the esters may be converted to ALA by nonspecific esterases. Alternatively, the esters may be hydrolysed to ALA outside the cell. Indeed the skin, in particular, possesses a multitude of different enzymes by which topically applied drugs can be metabolised.[20]

Cell culture studies have demonstrated that aliphatic straight chain ALA-esters, up as far as the hexyl ester in the homologous series, induce higher levels of PpIX in neoplastic cells more rapidly than the parent compound.[9,21,22] Surprisingly, few studies have investigated the penetration of ALA prodrugs into and across skin *in vitro*. The majority of these studies reveal that increased amounts of ALA prodrugs, relative to the parent compound, only penetrate the *stratum corneum* after prolonged application times.[23–25] Working within a framework of clinically relevant application times, such as 4 or 6 h, no significant difference is observed in amounts of ALA or ALA prodrugs penetrating *stratum corneum*.

It is important to note that many studies investigating ALA-esters have compared formulations *in vitro* and *in vivo* by using preparations containing the same amount of drug in terms of % w/w.[23–30] 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.

The aim of this study was to determine the influence of lipophilicity on ALA and ALA-ester penetration into skin using a range of *in vitro* and *in vivo* models. Importantly, ALA derivatives were incorporated into semisolid preparations at *equimolar* concentrations.

MATERIALS AND METHODS

Chemicals

ALA hydrochloride salt was obtained from Crawford Pharmaceuticals (Milton Keynes, UK). Methyl-ALA (m-ALA) hydrochloride salt, acetyl acetone, formaldehyde 37% w/w solution in water, thionyl chloride and hexanol were supplied by Sigma–Aldrich (Dorset, 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 was obtained from PerkinElmer Life Sciences (Beaconsfield, Bucks, UK). Unguentum Merck® was obtained from Merck® (Darmstadt, Germany). Cuprophan® dialysis membrane sheets were supplied by Medicell International (London, UK). Sevofluran was obtained from Abbot (Ludwigshafen, Germany). Radiolabelled ALA solution, 3.7 MBq mL^{-1} was supplied by PerkinElmer Life Sciences. All other chemicals were of analytical reagent grade.

Synthesis of Hexyl-ALA and Radiolabelled Esters

ALA hexylester was synthesised by reacting ALA with hexanol, as described previously.[31] Briefly, a defined volume of hexanol (3.5 mL) was cooled in an methanol-ice bath (−10°C), to which 0.5 mL thionyl chloride was added 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, 30 mL diethyl ether added and the solution stored at −70°C for 2 h to ensure the ester had precipitated out. The precipitate was then filtered under vacuum, and