Stability of 5-Aminolevulinic Acid in Novel Non-Aqueous Gel and Patch-Type Systems Intended for Topical Application

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Received 6 July 2004; revised 17 November 2004; accepted 7 April 2005

Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/jps.20393

ABSTRACT: Aminolevulinic acid (ALA) stability within topical formulations intended for photodynamic therapy (PDT) is poor due to dimerisation to pyrazine-2,5-dipropionic acid (PY). Most strategies to improve stability use low pH vehicles, which can cause cutaneous irritancy. To overcome this problem, a novel approach is investigated that uses a non-aqueous vehicle to retard proton-induced charge separation across the 4-carbonyl group on ALA and lessen nucleophilic attack that leads to condensation dimerisation. Bioadhesive anhydrous vehicles based on methylvinylether-maleic anhydride copolymer patches and poly(ethyleneglycol) or glycerol thickened poly(acrylic acid) gels were formulated. ALA stability fell below pharmaceutically acceptable levels after 6 months, with bioadhesive patches stored at 5°C demonstrating the best stability by maintaining 86.2% of their original loading. Glycerol-based gels maintained 40.2% in similar conditions. However, ALA loss did not correspond to expected increases in PY, indicating the presence of another degradative process that prevented dimerisation. Nuclear magnetic resonance (NMR) analysis was inconclusive in respect of the mechanism observed in the patch system, but showed clearly that an esterification reaction involving ALA and both glycerol and poly(ethyleneglycol) was occurring. This was especially marked in the glycerol gels, where only 2.21% of the total expected PY was detected after 204 days at 5°C. Non-specific esterase hydrolysis demonstrated that ALA was recoverable from the gel systems, further supporting esterified binding within the gel matrices. It is conceivable that skin esterases could duplicate this finding upon topical application of the gel and convert these derivatives back to ALA *in situ*, provided skin penetration is not affected adversely. J Pharm Sci 94:1756–1771, 2005

Keywords: chemical stability; stabilisation; formulation; polymeric drug delivery systems; HPLC (high-performance/pressure liquid chromatography); bioadhesive; patch; non-aqueous gel

INTRODUCTION

Photodynamic therapy (PDT) combines the irradiance of visible light with administration of a sensitising drug to bring about selective destruction of neoplastic cells undergoing rapid metabolism.[1] The action of a light dose of suitable wavelength produces excitation of the photosensitiser within intracellular space.[2,3] Energy relaxation *via* the transition of molecular oxygen to its singlet state is believed to be the primary cytotoxic species that causes irreversible cellular damage.[4] The most commonly used agent in PDT is 5-aminolevulinic acid (ALA), which is a naturally occurring precursor in the biosynthetic pathway of haem. Elevated intracellular

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Journal of Pharmaceutical Sciences, Vol. 94, 1756–1771 (2005)

concentrations of haem exert a negative feedback control over its biosynthesis. Administration of excess exogenous ALA avoids this process and, in so doing, induces a back accumulation of protoporphyrin IX (PpIX) due to the limited capacity of ferrochelatase. This enzyme is the final biosynthetic step in the pathway and inserts iron into the PpIX molecule.[2,5,6] Accumulation is pronounced in rapidly proliferating cells, which not only have reduced ferrochelatase activity, but also enhanced porphobilinogen deaminase activity.[3,7,8] The end result is photosensitisation resulting from elevated intracellular PpIX concentrations within selected cell populations.

The stability of ALA in non-biological, aqueous systems, such as drug delivery vehicles with a pH of 6 and above, is notoriously poor. It has been shown to be dependent on pH, concentration, temperature and degree of oxygenation of the solution.[9–12] The reaction, shown in Figure 1a, proposed by Novo Rodriguez et al.,[13] illustrates the acid-base equilibria leading to formation of 3,6-dihydropyrazine 2,5-dipropionic acid (DHPY). Deprotonation of the amino group allows cyclisation with the ketone group of a neighbouring molecule.[14] Further oxidation leads to pyrazine-2,5-dipropionic acid (PY), considered to be the major degradation product in aerated solutions.[9]

Condensation reactions of NH_2 containing compounds with carbonyl compounds usually require an optimised amount of acid catalyst. The reaction mechanism is shown in Figure 1b and reveals that nucleophilic attack by the lone pair of electrons is only possible when the nitrogen is unprotonated. Similarly, it is enhanced when the carbonyl is protonated. Clearly, concentrations of these species are affected oppositely by pH and a maximum reaction rate is achieved only when both are present in sufficient numbers to give a reasonable reaction rate. Consequently, a reduction in pH enhances stability of ALA in solution by precluding cyclisation as the amino group is extensively protonated. This happens in spite of protonation on the carbonyl group making it susceptible to attack. Thus, Elfsson et al.[15] found that solutions of ALA, buffered to pH 2.35, were stable over a period of 37 days, even when stored at 50°C. Adjusting the pH to approximately 2.0 is not, however, appropriate to topical dosage form design as cutaneous irritancy is probable. Addition of ethylene diamine tetra acetic acid[12,15] and antioxidants[16] to ALA solutions has resulted in disappointing improvements in stability. Further, the ever expanding range of potential neoplasias that are being treated with PDT, such as oral cancer[17] and gynaecological neoplasias,[18] has necessitated new approaches for drug delivery to these more challenging sites.

The aim of this study is to evaluate a novel alternative to enhancing the stability of ALA that is more applicable to topical delivery systems. Inspection of the mechanism in Figure 1b suggests that formulation of ALA-containing, non-aqueous systems, where the acid catalyst is not

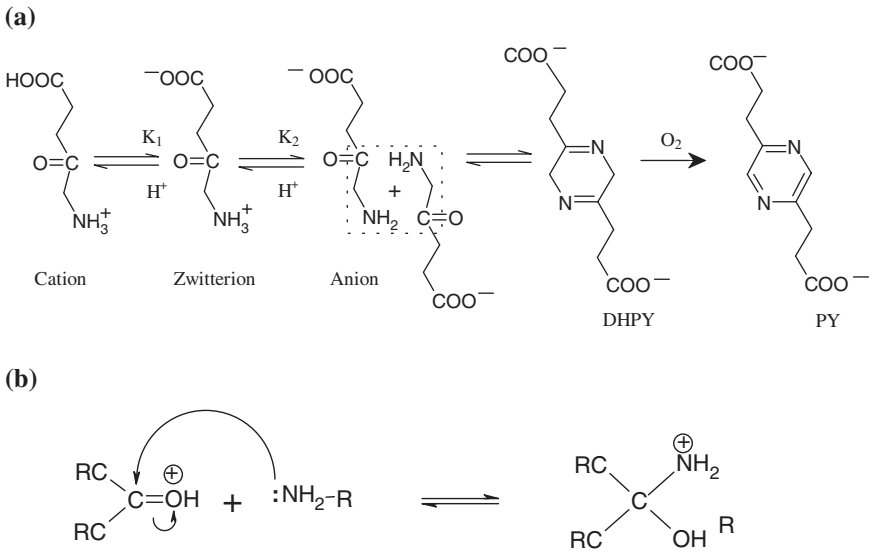

Figure 1. (a) pH-dependent equilibria occurring in aqueous solutions of 5-aminolevulinic acid (ALA). (b) Mechanism of nucleophilic attack by a primary amine on a carbonyl in the present of an acid catalyst.