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European Journal of Pharmaceutics and Biopharmaceutics 60 (2005) 439–444
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Topical glycerol monooleate/propylene glycol formulations enhance 5-aminolevulinic acid in vitro skin delivery and in vivo protophorphyrin IX accumulation in hairless mouse skin

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Received 8 March 2004; accepted in revised form 3 January 2005
Available online 7 April 2005

Abstract

Photodynamic therapy (PDT), a potential therapy for cancer treatment, utilizes exogenously applied or endogenously formed photosensitizers, further activated by light in an appropriate wavelength and dose to induce cell death through free radical formation. 5-Aminolevulinic acid (5-ALA) is a pro-drug which can be converted to the effective photosensitizer, protoporphyrin IX (PpIX). However, the use of 5-ALA in PDT is limited by the low penetration capacity of this highly hydrophilic molecule into appropriate skin layers. In the present study, we propose to increase 5-ALA penetration by using formulations containing glycerol monooleate (GMO), an interesting and useful component of pharmaceutical formulations. Propylene glycol solutions containing different concentrations of GMO significantly increased the in vitro skin permeation/retention of 5-ALA in comparison to control solutions. In vivo studies also showed increased PpIX accumulation in mouse hairless skin, after the use of topical 5-ALA formulations containing GMO in a concentration-dependent manner. The results show that skin 5-ALA penetration and PpIX accumulation, important factors for the success of topical 5-ALA-PDT in skin cancer, are optimized by GMO/propylene glycol formulations.

Keywords: 5-Aminolevulinic acid; Monoolein; Photodynamic therapy; Protoporphyrin IX; Skin cancer

1. Introduction

Photodynamic therapy (PDT) is a relatively new approach for the treatment of some kinds of cancer and non-malignant conditions [1,2]. The therapy involves topical or systemic administration of photosensitizers, followed by local application of adequate doses of light of appropriate wavelength in the presence of oxygen to induce tissue or cell destruction, presumably via formation of reactive oxygen species [3–5]. Conventional photosensitizers (hematoporphyrin derivatives) have a prolonged photosensitive effect after the treatment, due to the relatively slow clearance rate of these compounds from skin and certain other normal tissues. Thus, patients receiving standard dosages of these compounds must avoid exposure to sunlight for at least 2 weeks following administration. The inconvenience could be alleviated by the search for alternative compounds to be used in PDT [1].

Endogenous induction of photosensitizers is a recent development in tumor destruction through the topical or systemic application of 5-aminolevulinic acid (5-ALA) [1] a metabolic precursor of protoporphyrin IX (PpIX) in the biosynthetic pathway of heme [6]. The photodynamically active PplX can act as an endogenous photosensitizer [7] and it is almost completely cleared from the body within 24 h, reducing the risks of a general photosensitization for longer periods of time [4]. Systemically administered photosensitizers like hematoprophyrin or its more purified form

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doi:10.1016/j.ejpb.2005.01.011

Photofrin®-II, when used in clinical trials caused a generalized skin photosensitivity persisting for up to 8–10 weeks after treatment due to its relatively slow skin clearance rates [8,9]. Such serious side effects have intensified dermatologic research on topical application of sensitizers over the last 10 years. Studies in human volunteers and experimental animals have shown that 5-ALA-induced PpIX is almost completely cleared from the body within 24 h. Such rapid clearance lowers the risk of PpIX accumulation leading to prolonged photosensitivity, even when PDT treatment is repeated as often as every other day [10].

Due to the efficient photosensitizing effect of PpIX, 5-ALA has been experimentally used in clinical PDT for the treatment of some cancers like squamous cell carcinoma, actinic keratosis, basal cell carcinoma, Bowen's disease, as well as in diagnostic evaluations of skin, bladder, gastrointestinal tract and lung tumors [11,12].

Hydrophilic 5-ALA is a zwitterion at physiological pH which poorly crosses biological lipophilic barriers such as cell membranes. Crossing biological barriers is a vital condition for the conversion of therapeutically active 5-ALA to PpIX [13] and its improved skin penetration by pharmaceutical vehicles may play an important role in the success of PDT [4].

Several methodological or chemical proposals addressed this problem, like the use of iontophoresis [14,15], phonophoresis [16], conventional topical dosage forms [17], penetration enhancers [18,19], delivery systems [20–22] and 5-ALA derivatives [23,24], but there is still a gap in the provision of pharmaceutical preparations and methods to dermatologists for effective application in the topical PDT treatment of skin cancer [25].

One interesting strategy to be explored in topical PDT is the use of penetration enhancers [26], which reversibly reduce the barrier resistance of the stratum corneum (SC) and present low skin toxicity.

Monoolein is a mixture of glycerides and other fatty acids, mainly glycerol monooleate (GMO). It is biodegradable, nontoxic, biocompatible, generally recognized as safe [27,28], and has attracted great interest in the pharmaceutical area as a penetration enhancer in controlled drug delivery, bioadhesive systems and others [29]. It has been shown that GMO/solvent systems can be effective penetration enhancers for lipophilic drugs and highly polar compounds [30], probably acting through a temporary and reversible disruption of the ordered lamellar structure of SC bilayers. The SC intercellular lipid media is further increasingly fluidized by the removal of ceramides [31].

Structurally similar to oleic acid, a well-known skin penetration enhancer for several drugs [32,33], GMO enhances the skin penetration of several compounds such as urea, indomethacin, and steroids [30,34]. In addition, delivery systems for 5-ALA and photosensitizers based on GMO/water cubic phase gels showed adequate behavior in loading and preserving photochemical stability for these drugs [35].

The optimization of 5-ALA in vitro skin delivery and in vivo PpIX accumulation in hairless mouse skin promoted by the GMO enhancer were considered in this study, as decisive factors for the success of PDT in the treatment of skin cancer.

2. Materials and methods

2.1. Chemicals

5-Aminolevulinic acid hydrochloride was from Sigma Chemical Co. (St Louis, MO, USA); Monoolein–Myverol 18-99, containing distilled glycerol monooleate was from Quest International (Norwich, NY, USA). All other chemicals and solvents were of analytical grade.

2.2. Animals

The in vitro, in vivo and partition coefficient studies were carried out in male hairless mice, 6–8 weeks old (strain HRS/J Jackson Laboratories, Bar Harbor, ME, USA). The animals were housed at 24–26 °C, exposed to daily 12:12-h light: dark cycles (lights on at 6 a.m.), and had free access to standard mouse chow and tap water. To reduce the stress associated with the experimental procedure, mice were handled daily for 1 week before experimentation. They were euthanized by carbon dioxide vapor. The protocols were in accordance with the guidelines of the University of Sao Paulo Animal Care and Use Committee (Authorization number: 04.1.995.53.9).

2.3. Fluorometric assay

5-ALA was determined after conversion to its fluorescent derivative by reacting with acetylacetone and formaldehyde. The resulting derivative was assayed spectrofluorometrically using a HITACHI-F4500 spectrofluorometer (Tokyo, Japan), with excitation at 378 nm and emission at 464 nm [19,36].

2.4. Partition coefficient of 5-ALA between isopropyl myristate/water ($K_{IPM/water}$)

5-ALA partition coefficients between isopropyl myristate (IPM) and water were determined using the shake-flask method. Aqueous solutions of 5-ALA (60 μg/ml) were shaken with an equal volume of IPM for 30 min, the optimal time found for partition equilibrium of the drug between IPM and water phases. After standing for 5 min, the supernatant was removed and the residue centrifuged for 10 min at 2000*g* [19]. The content of the aqueous phase was determined by the spectrofluorometric assay described above at time zero (C_0) and after shaking to ensure partition (C). $K_{IPM/water}$ was calculated as $(C_0 - C)/C$.