Characterization of BF-200 ALA: a nanoemulsion-based drug delivery system for ALA-PDT

Introduction

Topical photodynamic therapy is a highly recommended and efficacious therapeutic approach for cutaneous neoplasia. It relies on the combination of three components: a photosensitizer, molecular oxygen and light of specific wavelengths. The activation of this photosensitizer by light induces the formation of reactive oxygen species (ROS). If a sufficient amount of ROS is obtained, cell death is induced. Two essential factors for the efficacy of ALA-based photodynamic therapy are stability and epidermal penetration of the photosensitizer or its produg. A commonly used photosensitizer for topical PDT is protoporphyrin IX (PpIX), which is induced selectively in tumor cells by the topuc application of its metabolite precursor 5-aminolevulinic acid (ALA). Stability of topical ALA preparations is critical, as ALA has been shown to prove to be degradation in aqueous solutions and standard formulations. A kin consequence, classical ALA in solutions has a very limited stability and has to be used within hours to days after production. ALA can be stabilized in combination with a specialized vehicle. A proprietary nanoscale lipid vesicle formulation patented by Biofrontera Biocience GmbH skin formulates BF-200 ALA has been reported to increase ALA stability for at least 24 months. As nanoemulsions have been reported to enhance epidermal penetration, this property was investigated with BF-200 ALA in two different models. BF-200 ALA greatly improves epidermal penetration of ALA and nanoscale lipid vesicle ALA formation.

Methods

- The composition of BF-200 ALA was analyzed using electron microscopy
- The stability of BF-200 was analyzed using pharmaceutical assays
- Epidermal penetration was investigated in two ex-vivo skin models, a porcine full-thickness skin model and a human full-thickness skin model in a Froner type incubation chamber
- With both skin models, epidermal PpIX formation was investigated microscopically, while in the human skin model, PpIX was additionally quantified in tissue lysates

Results

Composition and stability of BF-200 ALA

Electron microscope columns of nanoemulsion prepared by homogenizing technique

- Nanoemulsion BF-200 shows a very homogeneous size distribution
- Due to its hydrophilic nature, ALA probably interacts with the polar head groups of the lipid molecules of BF-200
- ALA is stable in BF-200 ALA over 24 months at both 5°C and 25°C

The interaction of ALA with BF-200

- Phospholipid and ALA most likely interact electrostatically
- The polar head groups of phospholipid molecules tend to the outside of the nanoemulsion thereby preventing the degradation of ALA

Skin penetration experiments

A. In a porcine ex-vivo skin model (Mauch et al., 2010)

Penetration depth of ALA (BF-200 ALA) or 5-ALA in an ex vivo pig skin model. The same amounts of both active ingredients were used. Penetration was assessed by measuring PpIX fluorescence signal in tissue lysates. Each value is the mean of 42 measurements.

- Nanosystem BF-200 optimizes transport of 5-ALA across the stratum corneum
- Significantly deeper PpIX induction with this formulation compared to 5-ALA
- No PpIX induction below the basal membrane

B. Skin penetration in a human skin in vitro model (Schneitz et al., 2006)

In order to analyze the effect of BF-200 on human ex vivo skin, we developed a novel culturing chamber for human epidermal skin explants to be grown at the liquid-air interface. ALA skin samples from super-washed normal healthy skin were exposed to either BF-200 ALA or 200 ALA. In vitro human skin hydration and structural photosensitizer levels were assessed in tissue slides to analyze the stability amounts of ALA. All measurements were performed on the same day of application. The results show that BF-200 ALA was used PpIX concentrations in the tissue are up to four times higher. The effect of skin barrier disruption after 72, 12, and 24 h (p<0.05, **p<0.01, ***p<0.001), while 5-ALA 200 ALA is at 200 ALA.

C. Proposed model for BF-200 mediated drug penetration

- Nanosystem BF-200 enhances ALA penetration and ALA-mediated photodynamic therapy

Conclusions

- Nanoemulsion BF-200 ALA is a stable and penetrative-measuring and vaporizing system for the use with BF-200 ALA in PDT
- The combination of ALA with nanoscale lipid vesicle formulation (nanoemulsion) allows using ALA to full capacity
- Improved skin penetration and epidermal PpIX formation could be demonstrated in two independent models (porcine and human skin)
- BF-200 ALA has a long shelf life (24 months) and open stability (3 months)

References