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

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Introduction

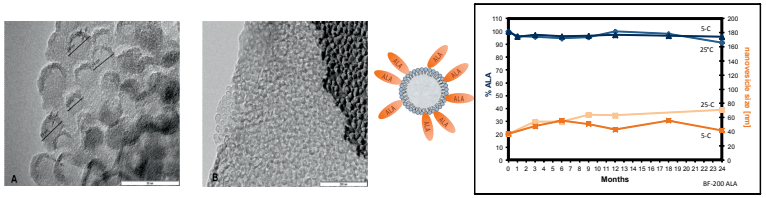
Topical photodynamic therapy is a highly recommended and efficacious therapeutic approach for cutaneous neoplasias. It relies on the combination of three components: a photosensitizer, molecular oxygen and light of a specific wavelength. The activation of this photosensitizer by light induces the formation of reactive oxygen species (ROS). If a sufficient amount of ROS is obtained, cell death is induced. Two essential factors for the efficacy ALA-based photodynamic therapy are stability and epidermal penetration of the photosensitizer or its prodrug. A commonly used photosensitizer for topical PDT is protoporphyrin IX (PpIX), which is induced selectively in tumor cell by the topical application of its metabolic precursor 5-aminolevulinic acid (ALA). Stability of topical ALA preparations is critical, as ALA has been shown to be prone to degradation in aqueous solutions and standard formulations. As a consequence, classical ALA in solution 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 Bioscience GmbH (also termed nanoemulsion BF-200) has been reported to increase ALA stability to 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 improved the epidermal penetration of ALA and subsequent PpIX 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 Trowell-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

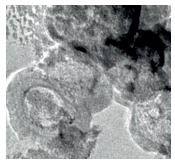


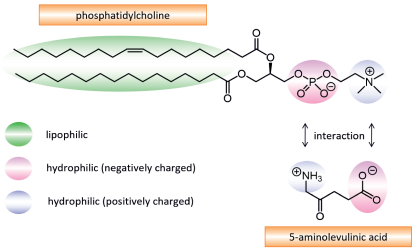
Electron microscopic pictures of nanoemulsion prepared by freeze-fracturing technique
Magnification (A) 1,200,000 fold (B) 250,000 fold

Stability study with BF-200 ALA.

- Nanoemulsion BF-200 shows a very homogeneous size distribution.
- Due to its hydrophilic nature, ALA probably interacts with the polar head groups of the lecithin 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

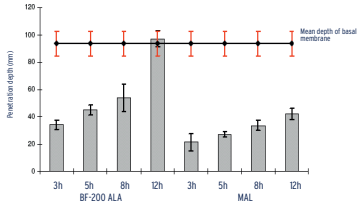




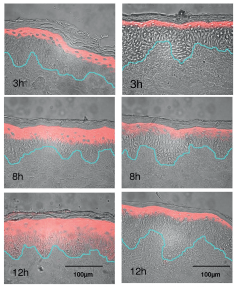
- Phosphatidylcholine and ALA most likely interact electrostatically
- The polar head groups of phospatidylcholines bind ALA to the outside of the nanovesicles thereby prevents the degradation of ALA

Skin penetration experiments

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



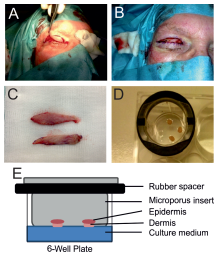
Penetration depth of ALA (in BF-200 ALA) or MAL in an ex vivo pig skin model. The same amounts of both active ingredients were used. Penetration was assessed by measuring PpIX fluorescence signals in tissue slices. Each value is the mean of 42 measurements.

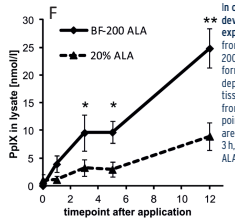


Distribution of UV-light induced Protopophyrin IX fluorescence in the upper layers of the pig skin (epidermis) after incubation with BF-200 ALA (left column) or MAL (right column) for 3, 8 and 12 hours. The fluorescence signal is shown in red. The blue line indicates the border between epidermis and dermis (basal

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

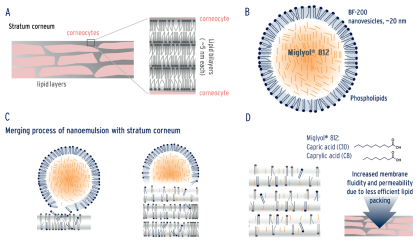
B. Skin penetration in a human skin in vitro model (Schmitz et al., 2016)





In order to analyze the effect of BF-200 on human ex vivo skin, we developed a novel culturing chamber for human upper eyelid skin explant to be grown at the liquid-air interface. A-E: Skin samples from upper eyelid surgery (healthy skin) were exposed to either BF-200 ALA or a 20% ALA ointment (non-ionic hydrophilic cream) PpIX formation was analyzed in tissue sections to measure penetration depth and intensity profiles. PpIX formation was also quantified from tissue lysates to analyze total amounts of PpIX. F: When measured from skin tissue lysates, PpIX concentrations are increased at all time points when BF-200 ALA was used PpIX concentrations in the lysates are up to four times higher. The effect is statistically significant after 3 h, 5 h and 12 h (*=p<0.05; **=p<0.01; U-test; n=8 BF-200 ALA; n=7 20% ALA).

C. Proposed model for BF-200 mediated skin penetration



Proposed mode of action for the nanoemulsion BF-200 and the human stratum corneum. A.) The human stratum corneum is composed of corneocytes embedded in lipids. These are packed in lipid bilayers of 5 nm thickness. B.) The BF-200 nanovesicles have a mean diameter of approximately 20 nm, are composed of a monolayer of phosphatidylcholine molecules, and are filled with a mixture of C8 and C10 fatty acid molecules (Miglyol® 812). C.) Upon contact with the stratum corneum lipid layers, the nanovesicle is presumed to undergo a fusion process. Within this process the phospholipids integrate into the lipid bilayers and the vesicle content is able to intersperse with the membrane lipids. D.) The shorter chain fatty acids integrate into the stratum corneum bilayers and cause a less efficient packing of the membrane. This increases membrane fluidity, which finally allows for easier penetration of ALA.

Conclusions

- Nanoemulsion BF-200 constitutes a stabilizing and penetration-enhancing formulation for the use with BF-200 ALA in PDT
- The combination of ALA with a nanoscale lipid vesicle formulation (nanoemulsion) allows using ALA to its 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

Reinhold U. A review of BF-200 ALA for the photodynamic treatment of mild-to-moderate actinic keratosis. Future Oncol. 2017 Aug 14. doi: 10.2217/fon-2017-0247.

Maisch T, Santarelli F, Schreml S, Babilas P, Szeimies RM. Fluorescence induction of protoporphyrin IX by a new 5-aminolevulinic acid nanoemulsion used for photodynamic therapy in a full-thickness ex vivo skin model. Exp Dermatol 19(8), e302-e305 (2010).

Schmitz L, Novak B, Hoeh AK, Luebbert H, Dirschka T. Epidermal penetration and protoporphyrin IX formation of two different 5-aminolevulinic acid formulations in ex vivo human skin. Photodiagnosis Photodyn Ther 14 40-46 (2016).