EXPERT OPINION

Liposomes in topical photodynamic therapy

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Introduction: Topical photodynamic therapy (PDT) refers to topical application of a photosensitizer onto the site of skin disease which is followed by illumination and results in death of selected cells. The main problem in topical PDT is insufficient penetration of the photosensitizer into the skin, which limits its use to superficial skin lesions. In order to overcome this problem, recent studies tested liposomes as delivery systems for photosensitizers.
Areas covered: This paper reviews the use of different types of liposomes for encapsulating photosensitizers for topical PDT. Liposomes should enhance the photosensitizers' penetration into the skin, while decreasing its absorption into systemic circulation. Only few photosensitizers have currently been encapsulated in liposomes for topical PDT: 5-aminolevulinic acid (5-ALA), temoporfin (mTHPC) and methylene blue.
Expert opinion: Investigated liposomes enhanced the skin penetration of 5-ALA and mTHPC, reduced their systemic absorption and reduced their cytotoxicity compared with free drugs. Their high tissue penetration should enable the treatment of deep and hyperkeratotic skin lesions, which is the main goal of using liposomes. However, liposomes still do not attract enough attention as drug carriers in topical PDT. *In vivo* studies of their therapeutic effectiveness are needed in order to obtain enough evidence for their potential clinical use as carriers for photosensitizers in topical PDT.

Keywords: 5-aminolevulinic acid, liposome, temoporfin, topical photodynamic therapy, vesicle

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1. Photodynamic therapy

Photodynamic therapy (PDT) represents a minimally invasive therapeutic approach used for various neoplastic and non-neoplastic diseases [1-3]. The advantages of PDT over conventional therapies are that it is minimally invasive, lacks systemic toxicity, shows selectivity, has a better functional and cosmetic outcome and can be repeated many times at the same site [2,3].

PDT requires the presence of a photosensitizing agent, oxygen and light of a specific wavelength which matches the absorption characteristics of the photosensitizer [1-3]. This combination leads mainly to the formation of the highly cytotoxic singlet oxygen (type 2 photo-oxidation, dominant mechanism), but also to generation of reactive oxygen species (ROS) (type 1 photo-oxidation), which cause tumor cell death [1-3].

Due to the drawbacks of the first-generation photosensitizers (such as porfimer sodium (Photofrin®, Axcan Pharma, Birmingham, AL, USA)), that is, long period of photosensitivity and limited depth of light penetration into tissue, second-generation photosensitizers have been developed [3]. These photosensitizers, including chlorines, purpurins, phthalocyanines, texaphyrins and 5-aminolevulinic acid (5-ALA) produce shorter periods of photosensitivity, have longer activation wavelengths and thus increased depth of effect (except 5-ALA), higher quantum yields of singlet oxygen and higher tumor to normal tissue concentrations [3,4].

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Article highlights

- 5-Aminolevulinic acid (5-ALA) has been almost exclusively used for topical photodynamic therapy (PDT) of skin diseases (actinic keratoses (AK), Bowen's disease (BD), basal-cell carcinoma (BCC), acne, viral warts, etc.).
- 5-ALA due to its hydrophilic and charge characteristics shows poor penetration into the skin, which limits the use of 5-ALA-PDT to only superficial malignant and non-malignant skin diseases; in favor to this is also the fact that the penetration depth of red light, used to activate 5-ALA (λ = 630 nm) into the skin is limited.
- Temoporfin (mTHPC) is activated with red light of longer wavelength (λ = 652 nm), which overcomes the problem of limited light penetration into tissue associated with 5-ALA-PDT; however, mTHPC shows also poor penetration into the skin due to its high molecular weight and extremely hydrophobic characteristics.
- The use of liposomes as delivery systems for 5-ALA and mTHPC enhances photosensitizers penetration and accumulation in the skin compared with that of the free photosensitizer, which should lead to higher PDT efficacy and enable topical PDT of deep and hyperkeratotic skin lesions.
- Liposomes reduce the absorption of the photosensitizer into systemic circulation compared with the free drug minimizing the risk of generalized photosensitivity.
- In most studies, liposomal 5-ALA induced higher protoporphyrin IX (PpIX) synthesis than free 5-ALA.
- As liposomes have shown to be a promising tool for delivering photosensitizers in topical PDT, they are supposed to attract more attention in foreseeable future.

This box summarizes key points contained in the article.

2. Topical PDT of the skin

For most indications the photosensitizer is administered systemically by intravenous injection [4]. In the case of cutaneous malignant (basal-cell carcinoma (BCC), squamous-cell carcinoma (SCC), Bowen's disease (BD), actinic keratoses (AK), etc.) or non-malignant diseases (psoriasis, acne, viral warts, etc.), a topical application of the photosensitizer onto the site of disease followed by illumination would be advantageous. Topical PDT would simplify the therapy, since the skin is readily accessible for topical treatment, increase the drug concentration in the skin, enhance patient compliance and restrict the residual photosensitivity only to the site of application. Topical PDT showing beneficial cosmesis (no scar formation) and no functional impairment would be an attractive treatment option for BCC, which arises on sun-exposed skin areas (such as the face) and is the most common cancer in white population, causing considerable patient morbidity [5]. Guidelines for topical PDT recommend its use in AC, BD, superficial and thin nodular BCC, as it shows recurrence rates equivalent to standard therapies, while its use in nodular BCC is less effective than surgery [6]. There is insufficient evidence to support the use of PDT in invasive SCC and in psoriasis, but there is an accumulating evidence base showing its high potential in treating inflammatory acne (although an optimized protocol is required), viral and genital warts and photorejuvenation [6].

However, the major obstacle in topical PDT, especially in PDT of thicker lesions (such as in nodular BCC), is the low permeability of the most apical layer of the skin, the stratum corneum (SC), to exogenous molecules (such as drugs). The low penetration of photosensitizers into the skin results in low doses reaching the site of action and therefore limited local activity [6,7]. In addition to limited photosensitizers' penetration into the skin (to only 1 – 2 mm depth [7,8]), the penetration depth of the red light into the skin is limited [3]. As a consequence, therapeutic effectiveness of PDT is reduced in BCC with lesions thicker than 2 mm [9,10] and the use of topical PDT is limited only to superficial skin lesions.

Skin penetration of photosensitizers can be increased by the use of different techniques developed to overcome the barrier properties of the SC [11-13]. They include the use of chemical penetration enhancers (CPEs) [13], different drug carrier systems [14-16], iontophoresis [17], sonophoresis [18], electroporation [19], microporation [20] and others. The problem of inefficient skin delivery of photosensitizers, as well as limited light penetration into the tissue, can be solved by pre-PDT deep curettage of the tumor in BCC, leading to high complete response rate in nodular (82 – 93%) and superficial (91 – 97%) BCC [9,21,22]. The limited penetration depth of light (used to activate the photosensitizer) into the skin can be overcome by the use of light of longer wavelengths to activate the same photosensitizer, as some photosensitizers have several absorption peaks, or by using photosensitizers that have longer activation wavelengths and thus increased depth of effect [4,6].

Topical PDT of superficial skin lesions has been, due to the high molecular weight of the aforementioned second-generation photosensitizers, mediated almost exclusively with semisolid dosage forms and emulsions containing 5-ALA or its methylester (MAL), which is more lipophilic and may penetrate more deeply into lesions [6,23,24].

3. Liposomes in enhancing drug penetration

Among different penetration enhancing methods, liposomes have been extensively studied for topical drug delivery [25-27]. Liposomes are colloidal particles, typically consisting of phospholipids, cholesterol (CH) and other possible ingredients. These lipid molecules form one or more concentric bimolecular layers enclosing an equal number of aqueous compartments. Liposomes can encapsulate hydrophilic drugs within the aqueous regions, and lipophilic molecules within the lipid bilayers [25].

Increasing attention has been paid over the last few decades to the use of liposomes as delivery systems for photosensitizers in systemic PDT, which has become a common practice, as

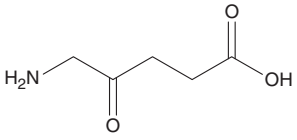

Figure 1. Chemical structure of 5-aminolevulinic acid (5-ALA).

liposomes improve the PDT efficacy as well as the photosensitizers' safety and selectivity [28]. In recent years, liposomes have been studied also as carrier systems for photosensitizers in topical PDT, since liposomes generally have the potential to enhance drug penetration into the skin [14,29], improve therapeutic effectiveness of the drug [30], decrease side effects [30] and act as a local depot for sustained release of active components [31]. As most photosensitizers are hydrophobic, liposomes serve also to solubilize them [28]. Depending on their composition, liposomes (i.e., vesicles) may be used for dermal (topical) and transdermal drug delivery [26].

The key parameter affecting the drug permeation across the SC and the interactions with the SC is the thermodynamic state of the vesicles' bilayers. Bilayers of vesicles are, depending on their lipid composition, either in a liquid crystalline, which is characterized by the fluid state of bilayers, or in a gel thermodynamic state, which is characterized by rigid bilayers. Thus, 'conventional' or 'traditional' liposomes, which are composed only of lipids (e.g., different phospholipids, CH, etc.), can be in fluid (liquid) or rigid (gel) thermodynamic state. Liposomes of liquid thermodynamic state have been proven to be superior to liposomes of gel thermodynamic state in terms of enhanced drug penetration [14,32,33]. Recently, a series of novel liposomes, that is, liquid-state vesicles with elastic (deformable) bilayers were developed, which have been shown to be superior to both gel and even liquid thermodynamic state conventional liposomes regarding interactions with human skin [34,35] and enhanced drug penetration [14,25,36,37].

Elastic vesicles include Transfersomes® (Idea AG, Germany), that is, vesicles containing besides phosphatidylcholine (PC) also edge activators (e.g., sodium cholate, polysorbate 80 or polysorbate 20) in their bilayers, which impart deformability to vesicles, being responsible for improved drug delivery [36,38,39]. Further, elastic vesicles are also vesicles composed of the bilayer-forming surfactant L-595 (sucrose laurate ester) and the micelle-forming surfactant PEG-8-L (octaoxyethylene laurate ester) [34], as well as ethosomes (as termed by the inventors [40]) containing phospholipids and high amounts of ethanol which increase the vesicles fluidity and deformability [39-41]. Recently, invasomes were introduced, which contain besides PC ethanol and terpenes being responsible for their high fluidity [42-44], and diethylene glycol monoethyl ether (Transcutol®)-containing vesicles, which have shown to be effective as dermal drug delivery systems [45]. In contrast to conventional liposomes, which can only be used for topical drug delivery, elastic vesicles have also been used for transdermal drug delivery [26].

The high penetration enhancing ability of deformable vesicles has been explained by their different mechanism of action compared with conventional liposomes. One proposed theory is that deformable vesicles penetrate as intact vesicles acting as 'carriers' for incorporated drugs. According to Cevc *et al.* [38], when Transfersomes are applied onto the skin under non-occlusive conditions, excess water starts to evaporate immediately. Then these ultradeformable and very hydrophilic aggregates begin to experience osmotic stress and the 'hydrational driving force' across the primary skin barrier in the SC builds up. Due to their high deformability and hydrophilicity, as well as the existing 'hydrational driving force', Transfersomes can overcome pores much narrower than their own diameter without changing their size significantly in order to avoid dry surroundings (skin surface) and to remain maximally swollen [38]. Hence, the drug-loaded ultradeformable vesicles are 'pulled' across the skin barrier along the transepidermal hydration gradient until they reach the viable epidermis where water abounds [38]. According to these authors, the vesicles penetrate intact into the deeper skin layers and even further [36,38,46]. Moreover, the same authors reported that intact vesicles penetrate into the SC through pre-existing channels with low penetration resistance [47]. This is supported by studies of Honeywell-Nguyen *et al.* [35]. However, Honeywell-Nguyen *et al.* [35] demonstrated partitioning of intact vesicles into the deeper layers of the SC, but not into the viable epidermis. The second theory proposed that elastic vesicles or vesicle constituents disorganize and disrupt intercellular lipid lamellae forming channel-like penetration pathways which are different from the aforementioned, through which drug molecules penetrate [34]. Regarding ethosomes, their inventors propose a synergistic effect of ethanol and vesicles on the skin lipids, where ethanol disturbs the organization of the SC lipid bilayers enhancing their fluidity, and it enhances the vesicles' fluidity. In addition, flexible ethosomes can penetrate the disturbed SC bilayers and even forge a pathway through the skin by virtue of their particulate nature [40]. A similar mechanism has been assumed for invasomes [42].

4. Photosensitizers used in topical PDT

4.1 5-Aminolevulinic acid

Topical PDT employing 5-ALA (**Figure 1**) has become a promising approach to treat superficial malignant and non-malignant skin lesions, such as BCC [21,48], AC [48-51], BD [48,52], psoriasis [23,53], viral warts [54,55] and acne [56].

5-ALA is the precursor of the potent endogenous photosensitizer protoporphyrin IX (PpIX) and on 5-ALA application cells generate PpIX through the heme biosynthetic pathway. Due to exposing cells to excess of exogenous 5-ALA, the first rate-limiting step in biosynthesis of heme (i.e., synthesis of ALA from succinyl-CoA and glycine by the enzyme

ALA synthetase) is bypassed, which causes synthesis and accumulation of PpIX [24]. Further, as the capacity of ferrochelatase to convert PpIX into heme (second rate-limiting step in heme biosynthesis) is limited, PpIX is accumulated in the cells [24]. PpIX represents a potent photosensitizer, activated on exposure to light with a wavelength ranging from 405 to 635 nm (blue to red light), with peak tumor fluorescence occurring 3 – 5 h after 5-ALA administration [3,50]. For tumor treatment the absorption peak of the longest wavelength (630 or 635 nm) is usually used for excitation due to highest tissue penetration [24]. The main advantage of PpIX compared with other photosensitizers is the short half-life of its photosensitizing effects, that is, PpIX is almost completely cleared from the body within 24 h [3,24]. As it gives no rise to cutaneous phototoxicity, it can be repeated often, which is especially important in palliative treatment. As PpIX is synthesized in mitochondria where it accumulates selectively, it is proposed that the primary cause of cell death after PDT is mitochondrial phototoxicity [24].

However, 5-ALA is a hydrophilic molecule with a molecular weight of 167.6 Da, octanol/water partition coefficient of 0.03 [57] and a SC/water partition coefficient of 0.1 [24]. Further, 5-ALA molecules are present as zwitterions at physiological pH, which carry a positive charge at the amine terminal and a negative charge at the carboxylic terminal. Such compounds show a limited capacity to reach and ultimately enter a target cell within a biological environment [58]. Therefore, the major limitation of PDT with 5-ALA is the poor penetration of 5-ALA through biological barriers like skin or cell membranes, due to its hydrophilic and charge characteristics [59]. Fortunately, 5-ALA-induced PpIX shows preferential accumulation in (pre)cancerous lesions, as well as in benign skin lesions (e.g., psoriasis and viral warts), which has been confirmed by fluorescence spectroscopy, fluorescence microscopy, fluorescence imaging and biochemical analysis [53,60,61]. This selectivity may be augmented by an increased permeability of the compromised barrier which is frequently associated with cutaneous diseases [62]. Using chronically UVB-exposed hairless mice skin as a skin model for (pre)cancerous lesions (since (pre)cancerous lesions may originate from chronically sun-exposed skin), it has been recently confirmed that the permeability properties of the SC overlying the (pre)cancerous lesions are indeed different from those of normal skin [63]. Thus, according to van den Akker *et al.* [63], the greater accumulation of PpIX in such lesions compared with the surrounding normal skin is the result, or at least partly the result, of a higher penetration rate of 5-ALA into (pre)cancerous lesions than into normal skin. However, debates still exist about the exact reason for the selective accumulation of 5-ALA in skin lesions. One of the reasons could be the lower levels of iron or reduced ferrochelatase activity in tumors compared with normal skin or a combination of both, which would lead to PpIX accumulation in tumors, since both are required for the conversion of PpIX to heme [64]. Due to higher 5-ALA penetration into (pre)cancereous lesions and hence selective PpIX accumulation and fluorescence, 5-ALA-PDT may also be used in the early detection of some malignancies [63].

Due to its low lipophilicity, 5-ALA does not show an effective penetration into hyperkeratotic lesions [48] and may even facilitate efflux via the microcirculation from deep nodular lesions [57]. Hence, the treatment of nodular or nodular-ulcerative tumors represents a problem in topical PDT with 5-ALA. In favor of this is the fact that despite PpIX being activated with the light of 630 nm wavelength (in order to maximize tissue penetration), this light still shows limited depth of tissue penetration [6,50]. Therefore, tumors deeper than 2 mm are not consistently cured. Thicker tumors can be successfully treated with more powerful photosensitizers activated at longer wavelengths, like temoporfin (mTHPC) (λ = 652 nm) [3,4].

5-ALA is usually applied in the form of 20% w/w oil-in-water emulsion or cream under occlusion [6,23,24]. In 1999, the US Food and Drug Administration (FDA) approved 5-ALA-PDT using Levulan® Kerastick, (20% 5-ALA hydrochloride topical solution, DUSA Pharmaceuticals, Wilmington, MA, USA) and the Blue-U® lamp for the treatment of AK of the face and scalp [6,50]. Later, MAL has also been approved as a 16% w/w cream (Metvix®, Photocure ASA, Norway and Galderma, France) for PDT of AK, BD and BCC [6].

Since low lipid solubility limits the clinical application of 5-ALA in thicker tumors or deeper skin lesions, different approaches are used to enhance the penetration of 5-ALA into the skin in order to enhance the PpIX accumulation in the skin [65]. Chemical methods include the use of 5-ALA esters [65], CPEs [24,60,66], microemulsions [67], cubic phase- [68] and sponge-phase delivery systems [69], liposomes [70,71] and others. Physical methods that have been used to enhance the 5-ALA skin penetration are: curettage (debulking) of nodular lesions [21], microdermabrasion [72], tape-stripping [61,62], sonophoresis [73], iontophoresis [17,74,75], microneedles [76] and needle-free jet injections [77]. Techniques that increase 5-ALA concentration in the target skin layers may improve the efficacy of topical PDT with this drug, since the PpIX accumulation is directly related to the presence of 5-ALA in the skin [24]. The results of only the most frequently used methods will be presented briefly in this review, as the purpose of present review is to concentrate on the use of liposomes.

Most *in vitro* results revealed that increased amounts of 5-ALA esters penetrate the SC only after long application times, and there was no significant difference between the amount of 5-ALA or 5-ALA esters penetrated *in vitro* into the SC after application times of 4 or 6 h (clinically relevant application times), regardless of the ester chain length (reviewed in [57]). Moreover, *in vivo* significant lag times were observed before PpIX fluorescence was induced by 5-ALA esters and they were longer than those induced by 5-ALA (reviewed in [57]). However, MAL has been shown to be effective in nodular BC [7] and it is approved as Metvix cream [6]. It has to be pointed out that in this study curettage/debulking was used before PDT in order

to remove the SC and some of the carcinoma. Regarding CPE, it has been reported that the association of 10% w/w 5-ALA with 3% w/w ethylenediaminetetraacetic acid disodium salt (Na_2EDTA) and 20% w/w dimethylsulfoxide (DMSO) enhanced *in vitro* the penetration of 5-ALA into the skin and increased by 2.5-fold the PpIX fluorescence *in vivo* [67]; the sentence should be: 10% w/w DMSO showed the highest enhancing effect among different enhancers (1-[2-(decylthio)-ethyl]azacyclopentan-2-one (HPE-101®), caprylic/capric triglyceride (Labrafac CC®), Labrafil®, caprylocaproyl macrogol-8 glycerides (Labrasol®) and Transcutol®) also for MAL [66].

As to the physical methods, pre-PDT deep curettage of tumors has already been mentioned to be successful in PDT of even nodular BCC [9,21,22]. Further, the transport of 5-ALA across the skin and its amount delivered into the skin were approximately fourfold greater with iontophoresis as compared with the passive 5-ALA application with added DMSO to the formulation [11]. Sonophoresis has also been reported to be successful in increasing the production of PpIX [73]. An important finding was that microneedle puncture could reduce the application time and the 5-ALA dose required to induce high levels of the photosensitizer protoporphyrin IX in the skin, these being notable advantages. However, puncture enhanced 5-ALA delivery to the upper skin regions, to mean depth of 1.875 mm [76].

In summary, the approach of using penetration enhancers in order to increase the 5-ALA skin penetration and accumulation of PpIX is feasible. However, penetration enhancers have yet to demonstrate enhanced therapeutic effectiveness in patients relative to conventional 5-ALA application, especially regarding PDT of deep skin tumors.

4.1.1 Liposomes as delivery systems for 5-ALA

The use of liposomes as potent delivery systems is expected to enhance the accumulation of 5-ALA in the deeper skin layers and, thus, the efficiency of PDT. Additionally, encapsulation into liposomes should enable the use of lower 5-ALA concentrations for PDT and thereby decrease the risk of skin photosensitzation, as well as the pain and pruritus associated with the use of high 5-ALA concentrations. Formulation issues should be kept in mind regarding the hydrophilic properties of 5-ALA, which make it difficult to entrap 5-ALA into liposomes with high entrapment efficiency [25]. This, however, might not be an obstacle, since Verma *et al.* [29] reported that liposomes enhanced the penetration of both encapsulated and non-encapsulated hydrophilic carboxyfluorescein into the skin. In addition, Barry [78] found no correlation between the entrapment efficiency and drug delivery. Consequently, there were many attempts to enhance the skin delivery of 5-ALA by using different types of liposomes, that is, conventional liposomes as well as novel elastic vesicles [70,71,79-83].

Pierre *et al.* [79] entrapped 5-ALA in liposomes having a lipid composition similar to the mammalian SC (stratum corneum lipid liposomes (SCLLs)). The *in vitro* 5-ALA permeation study showed that these liposomes increased the retention of 5-ALA in the target skin layers (dermis and epidermis without SC) compared with aqueous solution, while they decreased the amount of 5-ALA permeated through hairless mice skin. This favorable behavior can be explained as a consequence of the SCLLs–skin interaction, providing a deposit effect for 5-ALA in the skin [79]. SCLLs have already been proposed to act as skin 'drug localizers' due to their lipid composition [84].

Han *et al.* [80] entrapped 5-ALA in two types of liposomes, dimiristoyl-PC (DMPC) liposomes and glycerol dilaurate (GDL) liposomes to improve 5-ALA topical delivery. Both formulations increased PpIX expression in dorsal rat skin and pilosebaceous units compared with free 5-ALA. Moreover, the expression pattern and intensity of 5-ALA-induced PpIX in pilosebaceous units changed in a hair cycle-dependent manner.

Fang *et al.* [71] evaluated *in vitro* liposomes of various lipid composition (phosphatidylethanolamine (PE), PE/CH and PE/CH/sodium stearate (SS)) as carriers for 5-ALA, using intact rat skin, SC-stripped skin and delipidized skin. PE/CH/SS liposomes, being the best formulation, significantly increased 5-ALA skin penetration compared with other liposomes, and they provided an even 18-fold higher flux than that obtained with free 5-ALA. This was attributed to the presence of SS in the liposomes, which together with PE and CH exerts a penetration enhancing effect, while applied alone as a pretreatment followed by the application of 5-ALA aqueous solution, it did not enhance the penetration of 5-ALA. According to van Kuijk-Meuwissen *et al.* [33], phospholipids and/or surfactants being molecularly dispersed in the SC could act as penetration enhancers. In addition, it has been shown that the addition of dioleoylphosphatidylethanolamine (DOPE) [32] and surfactants (sodium cholate, polysorbates) [36,38] to liposomes enhanced the skin penetration of a fluorescence marker and drug, respectively. Additionally, confocal laser scanning microscopy (CLSM) [71], performed *in vitro* using sulforhodamine B as a fluorescent marker, confirmed the high potential of liposomes to deliver 5-ALA into the skin. The total fluorescence intensity of sulforhodamine B in the skin was 2.5-fold higher when it was encapsulated in PE/CH/SS liposomes compared with liposome-free formulations. Regarding the penetration depth, a broad distribution of red fluorescence that extended from the epidermis to the upper dermis was seen for sites treated with PE/CH/SS liposomes. The liposomes provided a maximum of fluorescence intensity at the depth of 72 – 96 μm, and the signal decreased from 108 μm to the skin bottom, showing its maximum possible depth at 180 μm of skin depth. These findings showing the potential of liposomes to deliver 5-ALA to the epidermis and dermis were encouraging and should lead in future to *in vivo* therapies using liposomal 5-ALA formulations.

Ethosomes, being more efficient vesicles, regarding the quantity and depth of drug penetration compared with either liposomes, aqueous or hydro-alcoholic solutions [39-41], were

also used as carriers for 5-ALA. Fang *et al.* [81] employed ethosomes of different composition (PE or PE/CH or PE/CH/SS and 15% ethanol) containing 5-ALA in order to enhance the skin production of PpIX. In an *in vivo* study in mice using CLSM, a higher PpIX-induced fluorescence was observed in the epidermis after the application of ethosomes compared with liposomes, indicating a greater penetration enhancing ability of ethosomes. The enhancement of PpIX intensity of all ethosome formulations was in the range of 11- to 15-fold in relation to that of the control (5-ALA in aqueous solution) and there was no correlation between the 5-ALA entrapment efficiency and PpIX accumulation in the skin.

Ethosomes loaded with 5-ALA were also investigated in a hyperproliferative murine skin model used to mimic diseased skin (i.e., psoriasis-like skin) with disordered SC and compromised barrier function [82]. In both the normal and diseased skin, it was shown that ethosomes significantly increased *in vitro* the skin delivery of 5-ALA and *in vivo* the fluorescence intensity of PpIX. The cumulative amounts of 5-ALA delivered by ethosomes *in vitro* in normal and hyperproliferative murine skin samples were 5- and 26-fold higher when compared with 5-ALA aqueous solution. The PpIX intensity in hyperproliferative murine skin obtained with 5-ALA-loaded ethosomes increased about 3.64-fold compared to that of the 5-ALA aqueous solution, while the maximal penetrated depth of PpIX was 30 μm and it extended to 80 μm (showing broad fluorescence distribution from 20 – 50 μm). These results indicated that PpIX remained in the lower epidermis and the upper dermis, or more precisely, only in the epidermis, as the epidermis is twofold thicker in hyperproliferative skin than in normal skin. Since most skin diseases, such as BCC and SCC, are located in the lower epidermis, this study confirmed the ability of ethosomal carriers to deliver 5-ALA to the target. Further, the ethosomes did not decrease the cell viability *in vitro* despite containing ethanol in contrast to the 5-ALA aqueous solution showing a slight decrease of the fibroblasts viability. In addition, the expression level of TNF-α, which plays an important role in psoriatic skin, was reduced after the application of 5-ALA-loaded ethosomes onto hyperproliferative murine skin, indicating that ethosomes recovered the skin.

Since ultradeformable vesicles have been shown to possess capability to carry the drug into the deep skin layers [36-38], Oh *et al.* [83] developed 5-ALA-loaded ultradeformable liposomes (containing polysorbate 20 in addition to lipids), with different surface charges and compared them with conventional neutral liposomes and solution. The cationic ultradeformable vesicles delivered *in vitro* the highest amount of 5-ALA into the viable hairless mice skin (dermis and epidermis without SC) and induced *in vivo* the highest accumulation of PpIX in the viable skin, which is an important prerequisite for an efficient topical PDT. This result is in accordance with the results obtained for cationic ultradeformable vesicles loaded with low-molecular-weight heparin [85,86] and with dexamethasone [87], showing higher drug delivery than neutral and anionic deformable vesicles, possibly because the cell surface of the skin bears a net negative charge [88]. Due to this fact, it was proposed that positive charges on the surface of cationic vesicles bind to the negative charges of the skin, thereby enhancing the penetration of liposomal particles into the skin. However, these results were inconsistent with the results obtained in studies of Manosroi *et al.* [89] and Ogiso *et al.* [90], where negatively charged (conventional) liposomes delivered the highest drug amounts into the deeper skin layers. Further, Kosobe *et al.* [91] reported that no significant changes were found in PpIX accumulation and PDT efficacy with increasing positive surface charges of liposomes. The higher effectiveness of cationic ultradeformable vesicles compared with conventional 5-ALA-liposomes can be explained by the presence of the surfactant polysorbate 20 in the lipid bilayers, which gives rise to the deformability of vesicles [36,38,92] enabling them to penetrate into pores smaller than their diameter (such as the pores in the SC) and carry the drug to the deep skin layers [92]. As the conversion of 5-ALA into PpIX occurs preferentially in the epidermis, these results suggested that cationic ultradeformable vesicles could optimize topical PDT with 5-ALA [83].

In contrast to the aforementioned publications, there are also some which did not report the superiority of liposomally encapsulated photosensitizers compared with free photosensitizers. Casas *et al.* [93] investigated the effects of topical application of 5-ALA in various formulations (saline lotion with and without DMSO, cream, conventional PC liposomes and vaseline) on the synthesis of porphyrins *in vivo* in a murine subcutaneous adenocarcinoma model. 5-ALA in saline lotion, alone or with 10% DMSO, proved to be the most efficient vehicle, whereas liposomes induced lower levels of porphyrin accumulation [93]. However, it should be kept in mind that liposomes of extremely large particle size were used (1 μm), which could limit the 5-ALA skin penetration and hence the porphyrin synthesis. Verma *et al.* [94] demonstrated *in vitro* in human skin that a small particle size of liposomes is necessary for an efficient drug delivery to the skin. Kosobe *et al.* [91] showed *in vitro* in cultivated cancer cells that the PDT efficacy increased with decreasing particle size of 5-ALA liposomes, and in particular liposomes smaller than 63.5 nm in diameter enhanced PDT efficacy compared with free 5-ALA.

Casas *et al.* [70] combined two approaches to improve the transmembrane access of 5-ALA, that is, they used both 5-ALA and its hexyl ester in their free form and encapsulated in conventional PC liposomes. It was found using tumor (M2 mammary adenocarcinoma) explant cultures that neither the use of hexyl ester nor the entrapment of either 5-ALA or hexyl ester into liposomes increased the rate of tumor porphyrin synthesis compared with that obtained with free 5-ALA [70], indicating no potential increase of PDT efficacy with the use of esters or liposomes. Moreover, exposure of the tumor cell line LM2 derived from this M2 mammary adenocarcinoma to liposomal 5-ALA or 5-ALA–hexyl ester produced less PpIX compared with their

free formulations [95]. This is in contrast with findings obtained *in vivo*, where 5-ALA-loaded liposomes induced an increased porphyrin accumulation in the tumor [96]. It should also be mentioned that liposomes used were of extremely large particle size (1 μm), which could limit their efficacy since Kosobe *et al.* [91] showed that the PDT efficacy increased with decreasing particle size of 5-ALA liposomes. However, employing the cell line LM2 it was found that for the synthesis of the maximum amount of porphyrins a 60-fold lower concentration of 5-ALA-hexyl ester was needed compared to that of 5-ALA [97]. These significant differences between results obtained with cell lines and parental tumors may be due to a large number of factors [97]. Regarding the use of the hexyl ester, it did not improve porphyrin synthesis either, which was instead significantly lower compared with the use of 5-ALA [70]. In addition, it has been shown that the exposure of tumor explants to either free or liposomal 5-ALA followed by illumination induces the same type of subcellullar damage [70].

The undecanoyl ester of 5-ALA (Und-5-ALA) was designed as a lipophilic 5-ALA derivative to enhance its skin penetration ability [98]. Since, Und-5-ALA induced low porphyrin content after being applied topically onto the skin over the tumor, it was encapsulated into PC/phosphatidylglycerol (PG) or PC/phosphatidic acid (PA) liposomes. Liposomal Und-ALA induced lower intracellular porphyrin content compared with free ALA *in vitro*, although their total porphyrins contents (intracellular + media) were the same, due to induction of porphyrins release induced by liposomes [98]. Moreover, topical application of Und-5-ALA in same liposomes *in vivo* onto the skin over the mammary adenocarcinoma LM3 subcutaneously injected in mice induced equal total amount of tumor porphyrins as compared with free Und-5-ALA [98]. Thus, the use of Und-5-ALA liposomes did not improve porphyrin synthesis either *in vitro* or *in vivo*, due to a massive release of extracellular porphyrins and a poor cytoplasmatic release of the liposome content. These results explain the behavior of highly lipophilic 5-ALA derivatives and show the limitations of liposomes. Therefore, liposomes of special composition are desired, which would be able to prevent Und-5-ALA interaction with cellular membrane and to overcome intracellular porphyrin release. The high cytotoxicity of free Und-5-ALA at concentrations above 0.3 mM was overcome by the use of liposomes [98].

As to the therapeutic effectiveness of liposomally encapsulated 5-ALA, there are only a few case studies on their use in PDT of skin diseases. Regarding *acne vulgaris*, it has already been shown that topical PDT with a 10 - 20% 5-ALA cream is very successful [99-103]. However, PDT with such high 5-ALA concentrations shows side effects at skin and mucosae level (pain, itching, erythema, crustae and pustules, as well as the post-treatment photosensitivity) which limits its use [56,104]. Therefore, soy PC liposomes with a 40-fold lower 5-ALA concentration were introduced, and indeed PDT of *acne vulgaris* with this 0.5% 5-ALA liposomal spray (Ellipse Photo Spray, Ellipse A/S, Denmark) and intense pulse light (IPL) in combination with topical peeling agents was shown to be safe and efficacious, even in patients with acne recalcitrant to standard therapy [104]. The mean improvement in total acne lesions was high (68.2%), the post-treatment fluorescence was low (i.e., reduced risk of phototoxicity) and the side effects were minimal after liposomes application. The obtained effectiveness was comparable with the effectiveness of 20% 5-ALA creams [100,101,103], but surpassed those reported in 16% MAL-PDT studies [105].

An *et al.* [106] reported that PDT using soy PC liposomes loaded with 0.5% 5-ALA (Ellipse Photo Spray, Ellipse A/S) was able to improve inflammatory *acne* even without the use of peeling agents, that is, the mean reduction in acne grade at the end of the treatment was 43.2%, and the side effects were minimal. A similar study using this 0.5% 5-ALA liposomal spray with IPL (emitting wavelengths from 400 to 720 nm) reported reduced inflammatory facial *acne* in Asians, with no noticeable side effects and a low risk of post-treatment phototoxic effects [107]. The mean reductions in inflammatory lesions were 52% at 1 month and 65% at 6 months after treatment.

Christiansen *et al.* [108] studied in normal skin the fluorescence distribution patterns and found that the average skin fluorescence after 30 min of incubation time with a 20% 5-ALA cream was comparable with that after 1 h of spraying with the aforementioned liposomal 0.5% 5-ALA spray at 5-min intervals. Moreover, fluorescence decays linearly within 15 min after spraying and returns to baseline within 8 h [109]. Thus, the use of liposomally encapsulated 5-ALA allows the concentration of 5-ALA to be reduced by a factor of 40, while inducing the same skin fluorescence (without the need of occlusion), and it was hypothesized that the short duration of fluorescence using liposomes with low 5-ALA concentration may result in short duration of phototoxicity.

As to PDT of *acne* and liposomes, methylene blue was also encapsulated into liposomes and afterward formulated in a hydrogel. The obtained liposomal hydrogel selectively delivered the drug to sebaceous glands and was effective in PDT of mild-to-moderate *acne vulgaris*. After only two treatments (once a week), the reduction in the number of inflammatory acne was 83.3%, while the reduction in the number of non-inflammatory acne was 63.6%. After 12 weeks, 90% of the patients showed a moderate to marked improvement of treated *acne* and no serious side effects were recorded [110].

The reported successful use of PC liposomes (conventional) with lower 5-ALA concentration in *acne vulgaris* is assumed to be due to the ability of liposomes to enhance 5-ALA skin penetration and its accumulation in the pilosebaceous units. Liposomes have been successfully employed in the treatment of hair follicle- and sebaceous gland-associated disorders because of their potential to carry lipophilic and hydrophilic drugs (such as 5-ALA) into the pilosebaceous units [111-113]. Moreover, a lot of studies demonstrated high therapeutic effectiveness of liposomally encapsulated drugs in the treatment of *acne vulgaris* [114-116].

PDT with 5-ALA or MAL has proven to be effective also for the treatment of photoaging [117,118]. Guidelines for topical PDT recommend PDT for photorejuvenation [6]. In order to overcome the side effects associated with the use of 20% 5-ALA creams under occlusion, PDT with the aforementioned 0.5% 5-ALA liposomal spray (Ellipse Photo Spray, Ellipse A/S) and IPL was used for the reduction of periorbital and nasolabial wrinkles [119]. The average overall improvement was significant for both the periorbital and the nasolabial wrinkles (but higher for periorbital wrinkles) in patients with type 2 photoaging, and the overall improvement was scored as excellent by 47% of the volunteers, while no side effects were observed during the treatment and afterward. In another study, same soy PC liposomes loaded with 5-ALA induced a significant improvement in wrinkle reduction and skin texture, being equivalent to that obtained with a 20% 5-ALA cream, although with fewer side effects [109]. Thus, the use of these 0.5% 5-ALA liposomes composed of soy PC has shown to be safe and effective for the treatment of acne, as well as for reducing wrinkles.

4.2 Temoporfin

mTHPC (**Figure 2**) is one of the most potent second-generation synthetic photosensitizers for PDT in present use [3,120]. *In vivo* studies have shown that PDT of the murine RIF-1 tumor with porfimer sodium required a light dose 4 – 13 times higher when compared with mTHPC for a similar antitumor effect [121]. This higher cytotoxicity presumably would lead to shorter treatment times for the same effect. The very potent chlorine mTHPC (with a quantum yield of singlet oxygen of about 0.87) is activated at 652 nm wavelength, showing a depth of light penetration of at least 1 cm, which enables the treatment of larger solid tumors [2]. Animal studies confirmed high tumor selectivity of mTHPC showing a ratio of 20:1 of tumor to normal tissue concentration [122]. Further, the residual skin photosensitivity after the treatment with mTHPC lasts only 2 weeks [2]. The aforementioned characteristics make mTHPC an almost ideal photosensitizer.

mTHPC has been shown to be effective in PDT of the SCC of the head and neck, that is, in the treatment of early or recurrent oral carcinomas and for the palliative treatment of refractory oral carcinomas [4,2,123]. On the basis of these trials, mTHPC, clinically used as a formulation in ethanol and propylene glycol (Foscan®, Biolitec Pharma Ltd., UK), has been approved in 2001 in the EU, Norway and Iceland for the palliative treatment of patients with advanced head and neck cancer who have failed prior therapies and are unsuitable for radiotherapy, surgery or systemic chemotherapy [124]. Clinical trials have also reported mTHPC to be effective in PDT of primary non-melanomatous tumors of the skin of the head and neck [4]. For all the aforementioned treatments mTHPC was used intravenously. Due to its high hydrophobicity, mTHPC has also been incorporated into conventional (Foslip®, Biolitec AG, Germany) and pegylated (Fospeg®, Biolitec AG) liposomes, and intensively investigated for intravenous administration [125,126].

mTHPC is also an interesting candidate for topical PDT of cutaneous malignant and non-malignant diseases. Unfortunately, mTHPC has a molecular weight of 680 Da, and is a highly hydrophobic drug (octanol/water partition coefficient of 9.4 [127]), being practically insoluble in all aqueous media, while it is soluble in alcohol, acetone and ethyl acetate [124]. Thus, mTHPC exhibits low percutaneous absorption and there are no formulations with mTHPC for topical use at the market. Since, mTHPC has been applied only intravenously to date in PDT of skin cancers, there are data available only on the mTHPC amount required to be present in the tumor to induce its necrosis on illumination, after intravenous application of mTHPC. It was shown in human colon carcinoma HT29-bearing mice that the mTHPC amount in the subcutaneously located tumor, required to induce tumor necrosis, was in the range 0.105 – 0.050 ng mTHPC/mg wet tissue weight, depending on the light dose used in PDT, that is, the amount decreased with increasing the light dose from 10 to 50 J/cm^2 [128]. However, these reported mTHPC amounts were necessary to induce necrosis of the very invasive HT29 tumor, showing only medium sensitivity against PDT, but there are no available data on mTHPC amounts required for a positive PDT outcome in any skin disease being less invasive and more sensitive to PDT.

There are only few published studies on the topical use of mTHPC. The first investigated topical formulation of mTHPC was a 2% mTHPC gel formulation, which was applied in patients with BD and BCC. Surprisingly, a low pathological clearance rate was obtained with this mTHPC gel [129]. The authors suggested that the limiting factor was the application method or the formulation of mTHPC. Therefore, in the case of drugs which have unfavorable penetration characteristics (such as mTHPC), it is of crucial importance to select and develop a suitable carrier system which will positively affect drug release and increase percutaneous penetration.

4.2.1 *Liposomes as delivery systems for mTHPC*

Liposomes have been investigated as delivery systems for mTHPC for both systemic and topical PDT [42-44,125,126].

Regarding topical PDT, the encapsulation of mTHPC into liposomes and their application in a murine skin tumor model revealed significant selectivity between lesion and normal surrounding, high mTHPC amounts within lesions and undetectable levels of generalized photosensitivity [130].

Further, in another study mTHPC was incorporated into two different kinds of conventional liposomes in liquid thermodynamical state (each composed of non-hydrogenated soy lecithin, but with different PC content), which were tested *in vitro* in human skin for their penetration enhancing ability. These liposomes enabled a satisfactory penetration of mTHPC into the skin delivering amounts which are supposed

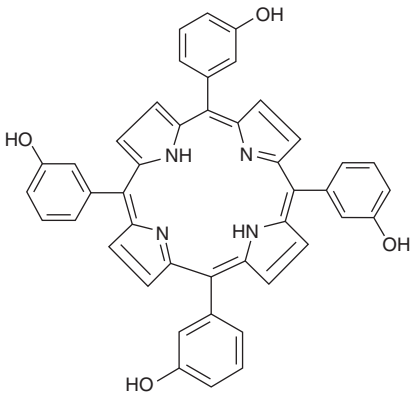

Figure 2. Chemical structure of temoporfin (mTHPC).

to be sufficient for a successful PDT [131]. Since the disadvantage of using liposomes topically, could be their liquid state of matter (i.e., they represent liquids which may leak from the application site), liposomes were further mixed with carbomer hydrogels of different viscosity (i.e., polymer content) in order to obtain semisolid formulations of mTHPC [131,132]. Obtained liposomal mTHPC-loaded gels of different polymer content (0.5, 0.75 and 1.0% w/w) decreased significantly (from 1.5- to 2.9-fold) the penetration of mTHPC into the skin compared with liposome dispersions [131]. The mTHPC-liposome gels showed an inverse relationship between the polymer content and mTHPC amount in the SC. The liposomal gel containing the mTHPC-loaded liposome dispersion (of higher PC content) and 0.75% w/w carbomer was considered to be the optimum formulation delivering an mTHPC amount into the SC and deeper skin layers, which was supposed to be sufficient for PDT, and showing also desirable rheological properties [131].

Since elastic vesicles are superior to conventional liposomes, elastic (deformable) vesicles named invasomes were used to enhance the skin penetration of mTHPC [42]. The first investigated mTHPC-loaded invasomes contained in addition to unsaturated soybean PC, also 3.3% w/v ethanol and 0.5 or 1% w/v of a terpene mixture composed of citral, cineole and D-limonene (cineole:citral:D-limonene = 45:45:10 v/v = standard mixture). The invasomes with 1% terpenes delivered a 2.1-, 3.8-, 2.3- and 1.6-fold higher amount of mTHPC (expressed as cumulative % dose applied/cm^2) to the skin compared with ethanolic solution, conventional liposomes, liposomes containing 3.3% ethanol and invasomes with 0.5% terpenes (showing a direct relationship between the amount of terpenes and the penetrated mTHPC amount), respectively. Obtained data revealed that invasomes containing 1% terpene mixture are highly effective in delivering the hydrophobic mTHPC into the SC and deeper skin layers being a prerequisite for a successful topical PDT [42]. The higher penetration enhancing ability of invasomes compared with conventional liposomes is proposed to be due to the presence of terpenes and ethanol in the carrier, which increase their fluidity and thus deformability being responsible for enhanced drug penetration. In order to further enhance the skin penetration of mTHPC, the same group of authors [43] varied the ratio between D-limonene, citral and cineole in the standard terpene mixture and used also single terpenes as invasomes constituents. As a result, seven new mTHPC-loaded invasome dispersions were developed. Among them, mTHPC-loaded invasomes containing 1% citral delivered the highest total amount of mTHPC to the skin, that is, a 4.6-fold higher amount than the conventional liposomes. However, in contrast to invasomes with 1% citral, which delivered a high amount to the SC, but a very small amount to the deeper skin layers, invasomes containing 1% cineole (delivering a 3.6-fold higher total amount of mTHPC than conventional liposomes) provided high amounts of mTHPC in the SC and also sufficient amounts in the deeper skin layers. This study showed that the incorporation of a single terpene into liposomes, in order to formulate invasomes, could also provide efficient nanocarriers for mTHPC. On the other hand, an important observation was that not all invasomes represent efficient delivery systems for mTHPC, that is, some of them (e.g., invasomes containing high amounts of D-limonene) did not provide sufficient mTHPC amounts in the skin for a successful PDT. Thus, the composition of the terpene mixture in invasomes determines the efficiency of the obtained invasome carrier system.

mTHPC-loaded invasomes containing 1% standard terpene mixture and invasomes with 1% citral were tested for their therapeutic effectiveness after topical application onto the skin of mice bearing the subcutaneously implanted human colorectal tumor HT29 followed by photoirradiation with 20 J/cm^2 [133]. The question was whether mTHPC-loaded invasomes can induce tumor necrosis or reduce tumor size by PDT or at least slow down tumor growth in mice compared with no treatment (control). mTHPC-loaded invasomes containing 1% standard terpene mixture significantly slowed down the tumor increase in mice during PDT not only compared with the control, but also compared with mTHPC-loaded invasomes with 1% citral and the ethanolic solution of mTHPC. However, invasomes were not able to reduce the size of the HT29 tumor. All features of the HT29 tumor (i.e., high invasiveness, intermediate sensitivity against PDT, subcutaneous location) limited the success of the therapy [133]. Despite the finding that invasomes do not provide sufficiently high mTHPC amounts in the subcutaneously located tumor being able to induce its necrosis, the results of this pilot study are very promising. They indicate the potential of invasomes to be used for the PDT of other skin diseases which are more sensitive to PDT, less invasive and for which there is no need for the drug to penetrate subcutaneously, like psoriasis, acne or different skin tumors (AK, BD, BCC). It is supposed that for these indications a smaller mTHPC amount would probably be sufficient for PDT, since different lesions do not require the same amount of photosensitizer to be present.

Moreover, mTHPC-loaded invasomes containing 1% standard terpene mixture, mTHPC-loaded invasomes containing

1% citral and mTHPC-ethanolic solution were tested for their PDT effectiveness *in vitro* in two cell lines, the tumor cell line HT29 and the epidermoid tumor cell line A431, in order to compare the sensitivity of these two carcinoma cell lines against PDT with these formulations [134]. The results revealed that mTHPC-loaded invasomes and mTHPC-ethanolic solution used at a 2 μM mTHPC concentration and photoirradiation with 20 J/cm^2 were able to reduce survival of HT29 cells and especially of A431 cells which are significantly more sensitive to PDT. In contrast to HT29 cells, where there was no significant difference between cytotoxicity of mTHPC-ethanolic solution and mTHPC-invasomes, in A431 cells mTHPC-invasomes were more cytotoxic. The 2 μM mTHPC-invasomes dramatically decreased the survival of A431 cells to about 16%, which was very promising, since it demonstrated invasomes' high potential for the use in topical PDT of cutaneous malignant diseases [134].

In addition to invasomes, mTHPC-loaded liposomes containing 3.3 - 20% w/v ethanol (i.e., ethosomes) were developed and evaluated *in vitro* in human skin for their penetration enhancing ability [135]. The increment of the amount of ethanol in mTHPC-liposomes increased the skin deposition of mTHPC. Conventional liposomes without ethanol (control) delivered the lowest absolute amount of mTHPC to the skin, while liposomes containing 20% ethanol delivered a 2.5-fold higher amount showing the highest penetration enhancement. The results indicated that these liposomes of high fluidity (due to high amounts of ethanol) could be a promising tool for delivering mTHPC to the skin in PDT of cutaneous diseases [135].

Chen *et al.* [44] found that most of mTHPC was accumulated in superficial skin layers on application of both invasomes and ethosomes, as well as of non-vesicular systems regardless if finite or infinite dose application was used.

Further, surface-charged flexible mTHPC-loaded liposomes which contain in the bilayers in addition to PC and other lipids (used to impart a surface charge to vesicles) the surfactant polysorbate 20, which provides deformability of vesicles, were developed as potential vehicles for mTHPC [136]. Neutral, anionic and cationic flexosomes induced a 2.2-, 1.9- and 2.6-fold higher mTHPC accumulation in the skin than conventional liposomes (expressed as cumulative % of the dose applied/cm^2), indicating a positive effect of the surfactant present in the membranes of liposomes on the penetration of mTHPC. They delivered amounts of mTHPC which are supposed to be sufficient for PDT not only to the SC, but also to the deeper skin layers [136]. The highest amount of mTHPC was delivered by cationic flexosomes to both the SC and deeper skin layers. However, there was no statistically significant difference in the penetration enhancing ability between these vesicles and the other flexosomes. This study implies that flexosomes, especially cationic flexosomes (due to their high stability), could be used as an efficient drug delivery system for the photosensitizer mTHPC.

Regarding the reviewed *in vitro* penetration studies on mTHPC, a very important finding was that mTHPC was not found in the acceptor compartment of the Franz cells during the *in vitro* penetration studies performed in human skin [42,43,131,135,136] regardless of the applied vesicle type, indicating no risk of systemic absorption of mTHPC and therefore no risk of systemic side effects, such as general photosensitivity. Further, it was obvious that all liposomes delivered significantly lower mTHPC amounts to the deeper skin layers than to the SC. However, this is not a drawback since mTHPC amounts delivered by selected invasomes, ethosomes and flexosomes are supposed to be sufficient for PDT according to previously mentioned unpublished results from Biolitec AG (Section 4.2 [128]).

Regarding therapeutic effectiveness, a topical 0.5 mg/ml mTHPC liposomal (dipalmitoyl-PC (DPPC)) thermosensitive gel formulation was studied in connection with PDT of non-pigmented skin malignancies (BCC and SCC) in humans, and no pain or swollen tissue or reddening of the treated area occurred during and after treatment, as is often seen in PDT using topical 5-ALA. One week after treatment, healing progress was observed in several patients and no complications were registered, indicating high therapeutic effectiveness of the used mTHPC-liposomal gel [137].

5. Conclusion

5-ALA has been almost exclusively used for topical PDT of superficial malignant and non-malignant skin diseases, while its efficacy in treating deeper skin lesions is limited. Thus, photosensitizers which would show higher therapeutic effectiveness in PDT of deep skin lesions were searched for, and in recent years a lot of attention has been paid to the use of mTHPC in PDT.

Unfortunately, both aforementioned photosensitizers exhibit unfavorable properties to penetrate the skin well and the selection/development of a suitable vehicle, which can affect drug release and percutaneous penetration, is in cases of such drugs of great importance.

The aim of this article was to review studies investigating the use of liposomes as carrier systems for 5-ALA and mTHPC. Most of the reviewed studies confirmed the ability of liposomes, both conventional liposomes and elastic vesicles, to enhance 5-ALA penetration into the skin and the accumulation of higher PpIX amounts in the target skin layers, inducing higher PpIX fluorescence compared with that of free 5-ALA. This is a prerequisite for a positive outcome of PDT, as the amount of PpIX in tumor tissue is directly correlated to the PDT efficacy. Moreover, the superiority of elastic vesicles over conventional liposomes in delivering the photosensitizer into the skin was reported *in vitro* and *in vivo*. In addition, it has been shown that the encapsulation of 5-ALA into liposomes decreases the cytotoxicity of 5-ALA. Regarding therapeutic effectiveness, soy PC liposomes enabled a 40-fold

reduction of the used 20% 5-ALA concentration, while ensuring the same clinical outcome in treating *acne vulgaris*.

Conventional liposomes and elastic vesicles also enhanced the skin penetration of mTHPC. Promising results were, however, obtained with elastic vesicles, that is, invasomes, ethosomes and flexosomes (vesicles containing polysorbate 20). The best results were obtained with invasomes, providing a high mTHPC amount in the SC and a smaller amount in the viable skin, which are assumed to be sufficiently high for PDT. As to the therapeutic effectiveness *in vivo*, mTHPC-loaded invasomes were unfortunately not able to decrease the size of the subcutaneously implanted HT29 tumor in mice, but they slowed down its increase compared with no treatment and treatment with the mTHPC-ethanolic solution. On the other hand, invasomes significantly decreased *in vitro* the survival of A431 carcinoma cells demonstrating their high potential to be used in PDT of cutaneous malignant diseases. This was a very promising finding indicating that mTHPC-loaded invasomes might be a promising tool for PDT of skin diseases which are more sensitive to PDT, less invasive than the HT29 malignant tumor and for which there is no need for the drug to penetrate subcutaneously, like psoriasis, acne or different superficial skin tumors.

Moreover, the mTHPC-liposomal thermosensitive gel has proven to be effective *in vivo* in PDT of non-pigmented skin malignancies in humans.

6. Expert opinion

The main problem in topical PDT is the insufficient photosensitizers' penetration into the skin, which limits its use to only superficial skin lesions. In order to overcome this problem, different penetration enhancement techniques have been used, especially for increasing the skin penetration of 5-ALA. Among these techniques, the use of various types of liposomes as delivery systems for photosensitizers has been explored in recent years. In contrast to systemic PDT, the use of liposomes in topical PDT is rather new and based on positive results obtained with both conventional liposomes and elastic vesicles as delivery systems for topical (dermal) drug delivery, such as enhanced drug penetration into the viable skin, accumulation of the drug at the site of action and reduction of systemic absorption. The high accumulation of the photosensitizer in the target skin layers would ensure a high PDT efficacy, while its reduced absorption into systemic circulation would decrease the risk of generalized photosensitivity. In contrast to conventional liposomes, elastic vesicles may also be used for transdermal drug delivery as they possess a higher penetration enhancing ability. Their penetration enhancing ability depends on their composition and the properties of the incorporated drug. However, since for topical PDT enhanced dermal drug delivery is desired (not transdermal drug delivery), conventional liposomes as well as elastic vesicles can be employed.

In addition to representing potent penetration enhancers, liposomes are harmless, that is, they are biodegradable, non-toxic, with low allergenic potential and generally recognized as safe (GRAS) by FDA, which is advantageous for topical PDT, since it is used in skin diseases, where the SC is already disordered and possesses decreased barrier properties. In addition, most of the vesicles investigated in the reviewed articles contain soy PC, which is rich in linoleic and linolenic acid, essential fatty acids possessing a critical role in the skin barrier function [138]. Linoleic acid has also been shown to be beneficial in the treatment of acne and even psoriasis (which are indications for PDT), since it restores the skin barrier function and reduces the scaling and epidermal hyperproliferation [138,139]. The aforementioned properties of liposomes represent an important advantage over other passive or active enhancement techniques, since they should be used in PDT in patients with acne, psoriasis and other benign or malignant skin diseases where the use of aggressive formulations/approaches could further worsen the condition of the diseased skin. CPEs are being intensively investigated as they may represent very potent enhancers for photosensitizers. However, they can cause skin irritancy, which is not surprising as they act by disrupting organized lipid structures in the SC and cell membranes. Thus, their toxicity often limits their usefulness for clinical application.

Further, an advantage of liposomes is also their simple and reproducible preparation and application to the skin. By contrast, electrically assisted methods (such as sonophoresis and iontophoresis) being very potent enhancers for 5-ALA, require specialized equipment, while curettage of tumors is usually not reproducible. The use of microneedles is, however, promising as it is minimally invasive and does not need advanced microelectronics. It will open a new frontier, in foreseeable future, for the delivery of drugs which cannot be delivered into/through the skin passively. However, at this stage there are hurdles to overcome in scaling up operations for the commercialization of their technology.

Keeping in mind the techniques used to enhance the skin penetration of photosensitizers, liposomes (due to their advantages) represent a promising approach.

The results reviewed in this manuscript indeed confirmed the ability of different liposomes to enhance the skin penetration of 5-ALA and mTHPC, reduce their systemic absorption and reduce their cytotoxicity compared with free drugs. It should be pointed out that elastic vesicles induced a significantly higher penetration of the photosensitizers into the skin than conventional liposomes. The high tissue penetration of the photosensitizers should enable PDT of benign and malignant skin diseases, especially of deep or hyperkeratotic skin lesions, which is the main goal of using both conventional liposomes and elastic vesicles. The reviewed studies confirmed the high effectiveness of 5-ALA liposomes in treating acne and photorejuvenation, and the ability of mTHPC-liposomes to slow down the increase of the tumor HT29 subcutaneously implanted in mice (but not to reduce

the tumor size) and to reduce significantly the cell viability of the epidermoid tumor cells A431 *in vitro* compared with other formulations. Unfortunately, there are no published results regarding the *in vivo* therapeutic effectiveness of liposomal photosensitizers in PDT of skin diseases (except of *acne*). Based on the reviewed results it can be assumed that PDT of superficial skin conditions could be feasible with certain types of investigated liposomes (especially elastic vesicles) loaded with 5-ALA and mTHPC. However, further studies investigating the effectiveness of liposomal photosensitizers are needed in order to give statements to whether or not PDT of deep skin lesions or hyperkeratotic skin lesions is manageable with these photosensitizer-loaded liposomes.

Thus, the main drawback of the reviewed studies is that most of them investigated *in vitro* the skin penetration of 5-ALA and mTHPC from different vesicles, as well as the production of 5-ALA-induced PpIX *in vivo*, while studies on their therapeutic effectiveness in skin diseases are rare. As to penetration studies, in most *in vivo* and *in vitro* studies healthy skin was used, which cannot predict precisely a drug's behavior in disordered skin. Therefore, instead of healthy skin, in future studies skin models should be used which mimic skin diseases, being potential indications for PDT. Regarding *in vitro* PDT effectiveness of 5-ALA and mTHPC-loaded liposomes, as it was tested only in few cell lines, future studies should involve different skin tumor cell lines and other relevant cell lines. *In vivo* studies of their therapeutic effectiveness are of crucial importance, and they are missing not only in humans, but also in animal models. These studies should be initially performed in relevant and established skin diesease (benign and malignant) models in animals, as well as in benign skin disease models (such as psoriasis) in animals. This has to be pointed out since the effectiveness of mTHPC-loaded invasomes was tested only in the HT29 tumor, which due to its high invasivity, as well as intermediate sensitivity against PDT and the subcutaneous location was not quite appropriate for evaluating their potential effectiveness in skin diseases. Afterward, PDT effectiveness of liposomal photosensitizers should be assessed *in vivo* in benign (especially psoriasis) and malignant (especially deep tumors) skin diseases in humans.

Keeping in mind all available data, there is no sufficient evidence on the therapeutic effectiveness of different vesicles loaded with mTHPC and 5-ALA in PDT of skin diseases to support their clinical use in topical PDT. *In vivo* studies are needed in order to obtain enough evidence for potential clinical use of liposomally encapsulated photosensitizers.

Stability issues may also be one of the reasons why liposomes are not extensively used in PDT. The shelf life of liposomes may be limited due to their potential physical or chemical instability, which is not rare. mTHPC-loaded invasomes, for example, are physically stable only when stored at 4°C [42]. In the case of 5ALA-loaded liposomes, there is a further concern as 5-ALA and its derivatives exhibit instability when they are not in acidic environment [140]. However, due to the risk of cutaneous irritation as well as instability of liposomes at pH values lower than 6.5 [141], liposomes containing 5-ALA and its derivatives (and generally their formulations) are usually not formulated at low pH values, which may also lead to short shelf lives. Thus, even conventional formulations like Porphin® cream (20% w/w 5-ALA cream, Crawford Pharmaceuticals, UK) and Metvix cream (16% w/w MAL) must be discarded 6 months after their production.

Additionally, different application doses of liposomes were used in the reviewed articles, which may significantly influence the results of the performed studies. This specifically refers to the studies with 5-ALA-loaded liposomes, since in the case of mTHPC-loaded vesicles the same finite dose (10 µl/cm^2 or 10 mg/cm^2) was used in most studies. Thus, the comparison of results from different studies on 5-ALA liposomes, as well as finding optimal doses, is very difficult.

A further very important reason why liposomes, despite their valuable properties confirmed in dermatopharmacotherapy (in the laboratory and in the clinic), have still not attracted enough attention for topical PDT as they should, could be the fact that PDT is managed by clinicians using conventional formulations (such as Levulan and Metvix) and not pharmaceutical technologists involved in the development of innovative drug formulations. Since the development or selection of a suitable vehicle for a specific drug is of great importance to attain the therapeutic aim, pharmaceutical technologists should be more involved in defining case studies in PDT.

To summarize, to date only few photosensitizers (5-ALA, mTHPC and methylene blue) have been investigated as encapsulated in different types of liposomes as potential tools for topical PDT. As the aforementioned findings are very encouraging, both conventional liposomes and elastic vesicles are supposed to enjoy in the near future a period of intense investigation as carriers for photosensitizers.

On one hand, these positive results will hopefully lead in future to the development of commercial liposomal formulations of mTHPC and 5-ALA for *in vivo* topical PDT of benign and malign skin diseases. However, in order to achieve this, future studies must be undertaken to fill in the gaps in the knowledge on the PDT therapeutic effectiveness of liposomes loaded with these photosensitizers in different skin diseases (especially in deep skin lesions). Currently, there is one liposomal formulation of 5-ALA available on the market (Ellipse Photo Spray, Ellipse A/S, Denmark) for the treatment of acne and wrinkles with the use of IPL.

On the other hand, these positive results should encourage future studies regarding development of different vesicles encapsulating other preformed photosensitizers (such as phthalocyanines) which are activated with light of even longer wavelength than mTHPC, showing an increased penetration depth into tissue.

In conclusion, as liposomes (especially elastic vesicles) were able to enhance the skin penetration of mTHPC and 5-ALA and may improve, thus, the PDT efficacy of photosensitizers,

they are supposed to be an objective of intense investigation in foreseeable future. This review highlights that mTHPC in addition to 5-ALA (and its methyl ester) could also be used for topical PDT.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Dougherty TJ, Gomer CJ, Henderson BW, et al. Photodynamic therapy. J Natl Cancer Inst 1998;90:889-905
2. Hopper C. Photodynamic therapy: a clinical reality in the treatment of cancer. Lancet Oncol 2000;1:212-19
3. Biel MA. Photodynamic therapy in head and neck cancer. Curr Oncol Rep 2002;4:87-96
4. Kubler AC, Haase T, Staff C. Photodynamic therapy of primary non-melanomatous skin tumours of the head and neck. Laser Surg Med 1999;25:60-8
5. Lien MH, Sondak VK. Nonsurgical treatment options for Basal cell carcinoma. J Skin Cancer 2011;2011:5717-34
6. Morton CA, McKenna KE, Rhodes LE; British Association of Dermatologists Therapy Guidelines and Audit Subcommittee and the British Photodermatology Group. Guidelines for topical photodynamic therapy: update. Br J Dermatol 2008;159:1245-66

• **A useful overview of current status of topical PDT.**

7. Peng Q, Soler AM, Warloe T, et al. Selective distribution of porphyrins in skin thick basal cell carcinoma after topical application of methyl 5-aminolevulinate. Photochem Photobiol B 2001;62:140-5
8. Ahmadi S, McCarron PA, Donnelly RF, et al. Evaluation of the penetration of 5-aminolevulinic acid through basal cell carcinoma: a pilot study. Exp Dermatol 2004;13:445-51
9. Rhodes LE, de Rie M, Enstrom Y, et al. Photodynamic therapy using topical methyl aminolevulinate vs surgery for nodular basal cell carcinoma: results of a multicenter randomized prospective trial. Arch Dermatol 2004;140:17-23
10. Mosterd K, Thissen MR, Nelemans P, et al. Fractionated 5-aminolaevulinic acid-photodynamic therapy vs. surgical excision in the treatment of nodular basal cell carcinoma: results of a randomized controlled trial. Br J Dermatol 2008;159:864-70
11. Barry BW. Breaching the skin's barrier to drugs. Nat Biotechnol 2004;22:165-7
12. Benson HA. Transdermal drug delivery: penetration enhancement techniques. Curr Drug Deliv 2005;2:23-33
13. Rizwan M, Aqil M, Talegaonkar S, et al. Enhanced transdermal drug delivery techniques: an extensive review of patents. Recent Pat Drug Deliv Formul 2009;3:105-24

• **A detailed overview of patents using different techniques to enhance skin penetration of drugs.**

14. El Maghraby GM, Williams AC, Barry BW. Skin delivery of 5-fluorouracil from ultradeformable and standard liposomes in vitro. J Pharm Pharmacol 2001;53:1069-77
15. Yuan JS, Yip A, Nguyen N, et al. Effect of surfactant concentration on transdermal lidocaine delivery with linker microemulsions. Int J Pharm 2010;392:274-84
16. Pardeike J, Hommoss A, Muller RH. Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. Int J Pharm 2009;366:170-84
17. Lopez RF, Bentley MV, Begona Delgado-Charro M, Guy RH. Optimization of aminolevulinic acid delivery by iontophoresis. J Control Release 2003;88:65-70
18. Cancel LM, Tarbell JM, Ben-Jebria A. Fluorescein permeability and electrical resistance of human skin during low frequency ultrasound application. J Pharm Pharmacol 2004;56:1109-18
19. Rastogi R, Anand S, Koul V. Electroporation of polymeric nanoparticles: an alternative technique for transdermal delivery of insulin. Drug Dev Ind Pharm 2010;36:1303-11
20. Bachhav YG, Heinrich A, Kalia YN. Using laser microporation to improve transdermal delivery of diclofenac: increasing bioavailability and the range of therapeutic applications. Eur J Pharm Biopharm 2011;78:408-14
21. Christensen E, Mørk C, Foss OA. Pre-treatment deep curettage can significantly reduce tumour thickness in thick Basal cell carcinoma while maintaining a favourable cosmetic outcome when used in combination with topical photodynamic therapy. J Skin Cancer 2011;2011:240340
22. Basset-Seguin N, Ibbotson SH, Emtestam L, et al. Topical methyl aminolaevulinate photodynamic therapy versus cryotherapy for superficial basal cell carcinoma: a 5 year randomized trial. Eur J Dermatol 2008;18:547-53
23. Ibbotson SH. Topical 5-aminolaevulinic acid photodynamic therapy for the treatment of skin conditions other than non-melanoma skin cancer. Br J Dermatol 2002;146:178-88
24. De Rosa FS, Bentley MV. Photodynamic therapy of skin cancers: sensitizers, clinical studies and future directives. Pharm Res 2000;17(12):1447-55

• **A detailed review on PDT in skin cancers.**

25. Choi MJ, Maibach HI. Liposomes and niosomes as topical drug delivery systems. Skin Pharmacol Physiol 2005;18:209-19

• **A very good review on the use of liposomes for topical drug delivery.**

26. El Maghraby GM, Williams AC. Vesicular systems for delivering conventional small organic molecules and larger macromolecules to and through human skin. Expert Opin Drug Deliv 2005;6:149-63

• **A comprehensive review on the use of liposomes for dermal/transdermal drug delivery.**

27. De Leeuw J, De Vijlder HC, Bjerring P, Neumann HA. Liposomes in dermatology today. J Eur Acad Dermatol Venereol 2009;23(5):505-16

28. Derycke ASL, De Witte PAM. Liposomes for photodynamic therapy. Adv Drug Deliv Rev 2004;56:17-30
• **An excellent review on the use of liposomes in PDT.**
29. Verma DD, Verma S, Blume G, Fahr A. Liposomes increase skin penetration of entrapped and non-entrapped hydrophilic substances into human skin: a skin penetration and confocal laser scanning microscopy study. Eur J Pharm Biopharm 2003;55:271-7
30. Seth AK, Misra A, Umrigar D. Topical liposomal gel of idoxuridine for the treatment of herpes simplex: pharmaceutical and clinical implications. Pharm Dev Technol 2004;9:277-89
31. Schmid MH, Korting HC. Therapeutic progress with topical liposome drugs for skin disease. Adv Drug Deliv Rev 1996;18:335-42
32. Kirjavainen M, Urti A, Jaaskelainen L, et al. Interaction of liposomes with human skin in vitro-the influence of lipid composition and structure, Biochim. Biophys Acta 1996;1304:179-89
33. Van Kuijk-Meuwissen MEMJ, Junginger HE, Bouwstra JA. Interactions between liposomes and human skin in vitro, confocal laser scanning microscopy study. Biochim Biophys Acta 1998;1371:31-9
34. Van den Bergh BAI, Vroom J, Gerritsen H, et al. Interactions of elastic and rigid vesicles with human skin in vitro: electron microscopy and two-photon excitation microscopy. Biochim Biophys Acta 1999;1461:155-73
• **A very good article investigating interaction of liposomes and the skin.**
35. Honeywell-Nguyen PL, De Graaff AM, Wouter Groenink HW, Bouwstra JA. The in vivo and in vitro interactions of elastic and rigid vesicles with human skin. Biochim Biophys Acta 2002;1573:130-40
• **A very good article investigating interaction of liposomes and the skin.**
36. Cevc G, Blume G. Biological activity and characteristics of triamcinolone-acetonide formulated with the self-regulating drug carriers, Transfersomes. Biochim Biophys Acta 2003;1614:156-64
37. Srisuk P, Thongnopnua P, Raktanonchai U, Kanokpanont S. Physico-chemical characteristics of methotrexate-entrapped oleic acid-containing deformable liposomes for in vitro transepidermal delivery targeting psoriasis treatment. Int J Pharm 2012;427(2):426-34
38. Cevc G, Mazgareanu S, Rother M, Vierl U. Occlusion effect on transcutaneous NSAID delivery from conventional and carrier-based formulations. Int J Pharm 2008;359:190-7
•• **An excellent article reporting therapeutic effectiveness of Transfersomes.**
39. Celia C, Cilurzo F, Trapasso E, et al. Ethosomes® and Transfersomes® containing linoleic acid: physicochemical and technological features of topical drug delivery carriers for the potential treatment of melasma disorders. Biomed Microdevices 2012;14:119-30
40. Touitou E, Dayan N, Bergelson L, et al. Ethosomes-novel vesicular carriers: characterization and delivery properties. J Control Release 2000;65:403-18
•• **An excellent article introducing ethosomes.**
41. Wo Y, Zhang Z, Zhang Y, et al. Preparation of ethosomes and deformable liposomes encapsulated with 5-fluorouracil and their investigation of permeability and retention in hypertrophic scar. J Nanosci Nanotechnol 2011;11:7840-7
42. Dragicevic-Curic N, Scheglmann D, Albrecht V, Fahr A. Temoporfin-loaded invasomes: development, characterization and in vitro skin penetration studies. J Control Release 2008;127:59-69
•• **First article published on the use of mTHPC encapsulated in liposomes for topical PDT and first detailed article on invasomes.**
43. Dragicevic-Curic N, Scheglmann D, Albrecht V, Fahr A. Development of different temoporfin-loaded invasomes-novel nanocarriers of temoporfin: characterization, stability and in vitro skin penetration studies. Colloids Surf B Biointerfaces 2009;70:198-206
44. Chen M, Liu X, Fahr A. Skin penetration and deposition of carboxyfluorescein and temoporfin from different lipid vesicular systems: in vitro study with finite and infinite dosage application. Int J Pharm 2011;408:223-34
45. Mura S, Manconi M, Valenti D, et al. Transcutol containing vesicles for topical delivery of minoxidil. J Drug Target 2011;19:189-96
46. Cevc G, Blume G. New, highly efficient formulation of diclofenac for the topical, transdermal administration in ultradeformable drug carriers. Transfersomes. Biochim Biophys Acta 2001;1514:191-205
47. Schatzlein A, Cevc G. Non-uniform cellular packing of the stratum corneum and permeability barrier function of intact skin: a high-resolution confocal laser scanning microscopy study using highly deformable vesicles (Transfersomes). Br J Dermatol 1998;138:583-92
•• **An excellent article introducing investigating penetration pathways in the skin.**
48. Dijkstra AT, Majoie IM, van Dongen JW, et al. Photodynamic therapy with violet light and topical 6-aminolaevulinic acid in the treatment of actinic keratosis, Bowen's disease and basal cell carcinoma. J Eur Acad Dermatol Venereol 2001;15:550-4
49. Jeffes EW, McCullough JL, Weinstein GD, et al. Photodynamic therapy of actinic keratoses with topical aminolevulinic acid hydrochloride and fluorescent blue light. J Am Acad Dermatol 2001;45:96-104
50. Jeffes EW. Levulan: the first approved topical photosensitizer for the treatment of actinic keratosis. J Dermatolog Treat 2002;13(Suppl 1):S19-23
51. Gold MH, Nestor MS. Current treatments of actinic keratosis. J Drugs Dermatol 2006;5(Suppl 2):S17-25
52. Morton CA, Whitehurst C, Moore JV, MacKie RM. Comparison of red and green light in the treatment of Bowen's disease by photodynamic therapy. Br J Dermatol 2000;143:767-72
53. Fritsch C, Ruzicka T. Fluorescence diagnosis and photodynamic therapy in dermatology from experimental state to clinic standard methods. J Environ Pathol Toxicol Oncol 2006;25(1-2):425-39
54. Stender IM, Na R, Fogh H, et al. Photodynamic therapy with 5-aminolaevulinic acid or placebo for recalcitrant foot and hand warts: randomised double-blind trial. Lancet 2000;355:963-6
55. Fabbrocini G, Di Costanzo MP, Riccardo AM, et al. Photodynamic therapy with topical delta-aminolaevulinic

acid for the treatment of plantar warts. J Photochem Photobiol B 2001;61:30-4

56. Sakamoto FH, Torezan L, Anderson RR. Photodynamic therapy for acne vulgaris: a critical review from basics to clinical practice: part II. Understanding parameters for acne treatment with photodynamic therapy. J Am Acad Dermatol 2010;63:195-211

57. Donnelly RF, McCarron PA, Morrow DI, et al. Photosensitiser delivery for photodynamic therapy. Part 1: topical carrier platforms. Expert Opin Drug Deliv 2008;5:757-66

•• **An excellent review on different topical carriers used for 5-ALA skin delivery.**

58. Fotinos N, Campo MA, Popowycz F, et al. 5-aminolevulinic acid derivatives in photomedicine: basics, application and perspectives. Photochem Photobiol 2006;82:994-1015

59. Peng Q, Berg K, Moan J, et al. 5-aminolevulinic acid-based photodynamic therapy: principles and experimental research. Photochem Photobiol 1997;65:235-51

60. van den Akker JT, Iani V, Star WM, et al. Topical application of 5-aminolevulinic acid hexyl ester and 5-aminolevulinic acid to normal nude mouse skin: differences in protoporphyrin IX fluorescence kinetics and the role of the stratum corneum. Photochem Photobiol 2000;72:681-9

61. Bogaards A, Aalders MC, Zeyl CC, et al. Localization and staging of cervical intraepithelial neoplasia using Double Ratio fluorescence imaging. J Biomed Opt 2002;7:215-20

62. Emtestam L, Nicander I, Stenstrom M, Ollmar S. Electrical impedance of nodular basal cell carcinoma: a pilot study. Dermatology 1998;197:313-16

63. van den Akker JT, Holroyd JA, Vernon DI, et al. Chronic UVB exposure enhances in vitro percutaneous penetration of 5-aminulevulinic acid in hairless mouse skin. Lasers Surg Med 2004;34:141-5

• **A very good article investigating the influence of skin barrier properties on 5-ALA accumulation in tumor.**

64. van den Akker JTHM, Brown SB. Photodynamic therapy based on 5-aminolevulinic acid: Applications in dermatology. In: Coohill TP, Valenzeno DP, editors. Photobiology for the 21st century. Valdenmar Publishing Co.; Kansas, USA: 2001. p. 165-81

65. Casas A, Batlle A. Aminolevulinic acid derivatives and liposome delivery as strategies for improving 5-aminolevulinic acid-mediated photodynamic therapy. Curr Med Chem 2006;13:1157-68

66. Bugaj A, Juzeniene A, Juzenas P, et al. The effect of skin permeation enhancers on the formation of porphyrins in mouse skin during topical application of the methyl ester of 5-aminolevulinic acid. J Photochem Photobiol B 2006;83:94-7

67. De Rosa FS, Marchetti JM, Thomazini JA, et al. A vehicle for photodynamic therapy of skin cancer: influence of dimethylsulphoxide on 5-aminolevulinic acid in vitro cutaneous permeation and in vivo protoporphyrin IX accumulation determined by confocal microscopy. J Control Release 2000;65(3):359-66

68. Turchiello RF, Vena FCB, Maillard PH, et al. Cubic phase gel as drug delivery system for topical application of 5-ALA, its ester derivatives and m-THPC in photodynamic therapy (PDT). J Photochem Photobiol B 2003;70:1-6

•• **The first reported use of cubic phase as a drug delivery system for PDT.**

69. Merclin N, Bender J, Sparr E, et al. Transdermal delivery from a lipid sponge phase–iontophoretic and passive transport in vitro of 5-aminolevulinic acid and its methyl ester. J Control Release 2004;100:191-8

70. Casas A, Perotti C, Saccoliti M, et al. ALA and ALA hexyl ester in free and liposomal formulations for the photosensitisation of tumour organ cultures. Br J Cancer 2002;86:837-42

71. Fang YP, Wu PC, Tsai YH, Huang YB. Physicochemical and safety evaluation of 5-aminolevulinic acid in novel liposomes as carriers for skin delivery. J Liposome Res 2008;18:31-45

72. Lee WR, Tsai RY, Fang CL, et al. Microdermabrasion as a novel tool to enhance drug delivery via the skin: an animal study. Dermatol Surg 2006;32:1013-22

73. Ma L, Moan J, Peng Q, Iani V. Production of protoporphyrin IX induced by 5-aminolevulinic acid in transplanted human colon adenocarcinoma of nude mice can be increased by ultrasound. Int J Cancer 1998;78:464-9

74. Mizutani K, Watanabe D, Akita Y, et al. Photodynamic therapy using direct-current pulsed iontophoresis for 5-aminolevulinic acid application. Photodermatol Photoimmunol Photomed 2009;25:280-2

75. Krishnan G, Roberts MS, Grice J, et al. Enhanced transdermal delivery of 5-aminolevulinic acid and a dipeptide by iontophoresis. Biopolymers 2011;96:166-71

76. Donnelly RF, Morrow DI, McCarron PA, et al. Microneedle-mediated intradermal delivery of 5-aminolevulinic acid: potential for enhanced topical photodynamic therapy. J Control Release 2008;129:154-62

•• **The first reported use of microneedles to enhance the skin penetration of a photosensitizer.**

77. Donnelly RF, Morrow DI, McCarron PA, et al. Influence of solution viscosity and injection protocol on distribution patterns of jet injectors: application to photodynamic tumour targeting. J Photochem Photobiol B 2007;89:98-109

•• **The first reported use of needle-free injectors to deliver a photosensitizer intradermally.**

78. Barry BW. Novel mechanisms and devices to enable successful transdermal drug delivery. Eur J Pharm Sci 2001;14:101-14

79. Pierre MB, Tedesco AC, Marchetti JM, Bentley MV. Stratum corneum lipids liposomes for the topical delivery of 5-aminolevulinic acid in photodynamic therapy of skin cancer: preparation and in vitro permeation study. BMC Dermatol 2001;1:5

80. Han I, Jun MS, Kim SK, et al. Expression pattern and intensity of protoporphyrin IX induced by liposomal 5-aminolevulinic acid in rat pilosebaceous unit throughout hair cycle. Arch Dermatol Res 2005;297:210-17

81. Fang YP, Tsai YH, Wu PC, Huang YB. Comparison of 5-aminolevulinic acid-encapsulated liposome versus ethosome for skin delivery for photodynamic therapy. Int J Pharm 2008;356:144-52

82. Fang YP, Huang YB, Wu PC, Tsai YH. Topical delivery of 5-aminolevulinic

acid-encapsulated ethosomes in a hyperproliferative skin animal model using the CLSM technique to evaluate the penetration behavior. Eur J Pharm Biopharm 2009;73:391-8

83. Oh EK, Jin SE, Kim JK, et al. Retained topical delivery of 5-aminolevulinic acid using cationic ultradeformable liposomes for photodynamic therapy. Eur J Pharm Sci 2011;44:149-57

84. Schmid MH, Korting HC. Liposomes: a drug carrier system for topical treatment in dermatology. Crit Rev Ther Drug Carrier Syst 1994;11:97-118

85. Song YK, Kim CK. Topical delivery of low-molecular-weight heparin with surface-charged flexible liposomes. Biomaterials 2006;27:271-80

86. Song YK, Hyun SY, Kim HT, et al. Transdermal delivery of low molecular weight heparin loaded in flexible liposomes with bioavailability enhancement: comparison with ethosomes. J Microencapsul 2011;28:151-8

87. Jain S, Jain P, Umamaheshwari RB, Jain NK. Transfersomes–a novel vesicular carrier for enhanced transdermal delivery: development, characterization, and performance evaluation. Drug Dev Ind Pharm 2003;29:1013-26

88. Burnette RR, Ongpipattanakul B. Characterization of the permselective properties of excised human skin during iontophoresis. J Pharm Sci 1987;76:765-73

89. Manosroi A, Kongkaneramit L, Manosroi J. Stability and transdermal absorption of topical amphotericin B liposome formulations. Int J Pharm 2004;270:279-86

90. Ogiso T, Yamaguchi T, Iwaki M, et al. Effect of positively and negatively charged liposomes on skin permeation of drugs. J. Drug Target 2001;9:49-59

91. Kosobe T, Moriyama E, Tokuoka Y, Kawashima N. Size and surface charge effect of 5-aminolevulinic acid-containing liposomes on photodynamic therapy for cultivated cancer cells. Drug Dev Ind Pharm 2005;31:623-9

92. Cevc G, Schatzlein A, Richardsen H. Ultradeformable lipid vesicles can penetrate skin and other semi-permeable membrane barriers unfragmented. Evidence from double label CLSM experiments and direct size measurement. Biochim Biophys 2002;1564:21-30

•• **A very interesting article discussing the mode of penetration of Transfersomes.**

93. Casas A, Fukuda H, Di Venosa G, Batlle AM. The influence of the vehicle on the synthesis of porphyrins after topical application of 5-aminolaevulinic acid. Implications in cutaneous photodynamic sensitization. Br J Dermatol 2000;143:564-72

• **An interesting article on the importance of the selection of a proper vehicle for a photosensitizer.**

94. Verma DD, Verma S, Blume G, Fahr A. Particle size of liposomes influences dermal delivery of substances into skin. Int J Pharm 2003;258:141-51

95. Cases et al. Unpublished results, cited in: Casas A, Perotti C, Saccoliti M, et al. ALA and ALA hexyl ester in free and liposomal formulations for the photosensitisation of tumour organ cultures. Br J Cancer 2002;86:837-42

96. Fukuda H, Paredes S, Batlle AM. Tumour-localizing properties of porphyrins. In vivo studies using free and liposome encapsulated aminolevulinic acid. Comp Biochem Physiol B 1992;102(2):433-6

97. Casas A, Fukuda H, Di Venosa G, Batlle A. Photosensitization and mechanism of cytotoxicity induced by the use of ALA derivatives in photodynamic therapy. Br J Cancer 2001;85:279-84

98. Di Venosa G, Hermida L, Fukuda H, et al. Comparation of liposomal formulations of ALA Undecanoyl ester for its use in photodynamic therapy. J Photochem Photobiol B 2009;96:152-8

99. Wang XL, Wang HW, Zhang LL, et al. Topical ALA PDT for the treatment of severe acne vulgaris. Photodiagnosis Photodyn Ther 2010;7:33-8

100. Gold MH. The utilization of ALA-PDT and a new photoclearing device for the treatment of severe inflammatory acne vulgaris – results of an initial clinical trial. J Lasers Surg Med 2003;15:46

101. Gold MH, Bradshaw VL, Boring MM, et al. The use of a novel intense pulsed light and heat source and ALA PDT in the treatment of moderate to severe inflammatory acne vulgaris. J Drugs Dermatol 2004;3:15-19

102. Gold MH, Biron JA, Boring M, et al. Treatment of moderate to severe inflammatory acne vulgaris: photodynamic therapy with 5-aminolevulinic acid and a novel advanced fluorescence technology pulsed light source. J Drugs Dermatol 2007;6:319-22

103. Goldman MP, Boyce S. A single-center study of aminolevulinic acid and 417 nm photodynamic therapy in the treatment of moderate to severe acne vulgaris. J Drugs Dermatol 2003;2:393-6

104. de Leeuw J, van der Beek N, Bjerring P, Neumann HA. Photodynamic therapy of acne vulgaris using 5-aminolevulinic acid 0.5% liposomal spray and intense pulsed light in combination with topical keratolytic agents. J Eur Acad Dermatol Venereol 2010;24:460-9

• **An interesting article on the use of liposomally encapsulated 5-ALA in acne vulgaris.**

105. Wiegell SR, Wulf HC. Photodynamic therapy of acne vulgaris using methylaminolaevulinate: a blinded, randomized, controlled trial. Br J Dermatol 2006;154:969-76

106. An JS, Kim JE, Lee DH, et al. 0.5% Liposome-encapsulated 5-aminolevulinic acid (ALA) photodynamic therapy for acne treatment. J Cosmet Laser Ther 2011;13(1):28-32

107. Yeung CK, Shek SY, Yu CS, et al. Liposome-encapsulated 0.5% 5-aminolevulinic acid with intense pulsed light for the treatment of inflammatory facial acne: a pilot study. Dermatol Surg 2011;37(4):450-9

108. Christiansen K, Bjerring P, Troilius A. 5-ALA for photodynamic photorejuvenation-optimization of treatment regime based on normal skin fluorescence measurements. Lasers Surg Med 2007;39:302-10

109. Bjerring P, Christiansen K, Troilius A, et al. Skin fluorescence controlled photodynamic photorejuvenation (wrinkle reduction). Lasers Surg Med 2009;41(5):327-36

110. Fadel M, Salah M, Samy N, Mona S. Liposomal methylene blue hydrogel for selective photodynamic therapy of acne vulgaris. J Drugs Dermatol 2009;8(11):983-90

111. Lieb LM, Ramachandran C, Egbaria K, Weiner N. Topical delivery enhancement with multilamellar liposomes into pilosebaceous units. I. In vitro evaluation

using fluorescent techniques with hamster ear model. J Invest Dermatol 1992;99:108-13

112. Lauer AC. Percutaneous drug delivery to the hair follicle. In: Bronaugh RL, Maibach HI, editors. Percutaneous absorption drugs-cosmetics-mechanisms-methodology. 3rd edition. Marcel Dekker, Inc.; New York, Basel: 1999. p. 427-49

113. Tabbakhian M, Tavakoli N, Jaafari MR, Daneshamouz S. Enhancement of follicular delivery of finasteride by liposomes and niosomes. 1. In vitro permeation and in vivo deposition studies using hamster flank and ear models. Int J Pharm 2006;323:1-10

114. Patel VB, Misra AN, Marfatia YS. Topical liposomal gel of tretinoin for the treatment of acne: research and clinical implications. Pharm Dev Technol 2000;5:455-64

115. Patel VB, Misra AN, Marfatia YS. Preparation and comparative clinical evaluation of liposomal gel of benzoylperoxide for acne. Drug Dev Ind Pharm 2001;27:863-70

116. Castro GA, Ferreira LA. Novel vesicular and particulate drug delivery systems for topical treatment of acne. Expert Opin Drug Deliv 2008;5:665-79

117. Zane C, Capezzera R, Sala R, et al. Clinical and echographic analysis of photodynamic therapy using methylaminolevulinate as sensitizer in the treatment of photodamaged facial skin. Lasers Surg Med 2007;39(3):203-9

118. Park MY, Sohn S, Lee ES, Kim YC. Photorejuvenation induced by 5-aminolevulinic acid photodynamic therapy in patients with actinic keratosis: a histologic analysis. J Am Acad Dermatol 2010; 62(1):85-95

119. Piccioni A, Fargnoli MC, Schoinas S, et al. Efficacy and tolerability of 5-aminolevulinic acid 0.5% liposomal spray and intense pulsed light in wrinkle reduction of photodamaged skin. J Dermatolog Treat 2011; 22(5):247-53

120. Mitra S, Foster TH. Photophysical parameters, photosensitizer retention and tissue optical properties completely account for the higher photodynamic efficacy of meso-tetra-hydroxyphenyl-chlorin vs Photofrin. Photochem Photobiol 2005;81:849-59

121. Van Geel IP, Oppelaar H, Oussoren YG, et al. Photosensitizing efficacy of MTHPC-PDT compared to photofrin-PDT in the RIF1 mouse tumour and normal skin. Int J Cancer 1995;60:388-94

122. Ris H-B, Altermatt JH, Stewart CM, et al. Photodynamic therapy with m-tetrahydroxyphenylchlorin in vivo: optimization of the therapeutic index. Int J Cancer 1993;55:245-9

123. Hopper C, Kubler A, Lewis H, et al. mTHPC-mediated photodynamic therapy for early oral squamous cell carcinoma. Int J Cancer 2004;111:138-46

124. Available from: www.biolitecpharma.com

125. Buchholz J, Kaser-Hotz B, Khan T, et al. Optimizing photodynamic therapy: in vivo pharmacokinetics of liposomal meta-(tetrahydroxyphenyl)chlorin in feline squamous cell carcinoma. Clin Cancer Res 2005;11:7538-44

126. Svensson J, Johansson A, Grafe S, et al. Tumour selectivity at short times following systemic administration of a liposomal temoporfin formulation in a murine tumour model. Photochem Photobiol 2007;83:1211-19

127. Kelbauskas L. Untersuchungen zur Struktur-Eigenschafts-Beziehung selbstassoziierender Photosensibilisatoren mittels zeitaufgeloster Spektroskopie. Friedrich-Schiller University Jena, Jena, Ph.D. Thesis; Germany: 2003

128. Biolitec AG. Jena, Germany, unpublished results cited in: Dragicevic-Curic N, Scheglmann D, et al. Temoporfin-loaded invasomes: development, characterization and in vitro skin penetration studies. J Control Release 2008;127:59-69

129. Gupta G, Morton CA, Whitehurst C, et al. Photodynamic therapy with meso-tetra(hydroxyphenyl)chlorin in the topical treatment of Bowen's disease and basal cell carcinoma. Br J Dermatol 1991;141:385-6

130. Johansson A, Svensson J, Bendsoe N, et al. Fluorescence and absorption assessment of a lipid mTHPC formulation following topical application in a non-melanotic skin tumour model. J Biomed Opt 2007;12:034026

131. Dragicevic-Curic N, Winter S, Stupar M, et al. Temoporfin-loaded liposomal gels: viscoelastic properties and in vitro skin penetration. Int J Pharm 2009;373(1-2):77-84

132. Dragicevic-Curic N, Winter S, Krajisnik D, et al. Stability evaluation of temoporfin-loaded liposomal gels for topical application. J Liposome Res 2010;20(1):38-48

133. Dragicevic-Curic N, Grafe S, Albrecht V, Fahr A. Topical application of temoporfin-loaded invasomes for photodynamic therapy of subcutaneously implanted tumours in mice: a pilot study. J Photochem Photobiol B 2008;91(1):41-50

134. Dragicevic-Curic N, Grafe S, Gitter B, Fahr A. Efficacy of temoporfin-loaded invasomes in the photodynamic therapy in human epidermoid and colorectal tumour cell lines. J Photochem Photobiol B 2010;101(3):238-50

135. Dragicevic-Curic N, Scheglmann D, Albrecht V, Fahr A. Development of liposomes containing ethanol for skin delivery of temoporfin: characterization and in vitro penetration studies. Colloids Surf B Biointerfaces 2009;74(1):114-22

136. Dragicevic-Curic N, Grafe S, Gitter B, et al. Surface charged temoporfin-loaded flexible vesicles: in vitro skin penetration studies and stability. Int J Pharm 2010;Jan15384(1-2):100-8

137. Bendsoe N, Persson L, Johansson A, et al. Fluorescence monitoring of a topically applied liposomal Temoporfin formulation and photodynamic therapy of nonpigmented skin malignancies. J Environ Pathol Toxicol Oncol 2007;26:117-26

138. Rhodes LE. Essential fatty acids. In: Loden M, Maibach HI, editors. Dry skin and moisturizers. Chemistry and Function; Boca Raton, London, New York: 2000. p. 311-25

139. Fluhr JW, Berardesca E. Effects of moisturizers and keratolytic agents in psoriasis. In: Loden M, Maibach HI, editors. Dry skin and moisturizers. Chemistry and Function; Boca Raton, London, New York: 2000. p. 167-72

140. McCarron PA, Donnelly RF, Andrews GP, Woolfson AD. Stability of 5-aminolevulinic acid in novel non-aqueous gel and patch-type systems intended for topical application. J Pharm Sci 2005;94:1756-71

141. Thoma K, Jocham UE. Liposome dermatics: assessment of long-term stability. In: Braun-Falco O, Korting HC, Maibach HI, editors. Liposome Dermatics. Springer-Verlag; Berlin: 1992. p. 150-66

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