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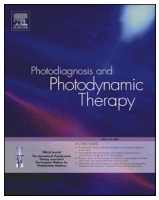

Review

Improving the efficacy of photodynamic therapy for actinic keratosis: A comprehensive review of pharmacological pretreatment strategies

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ABSTRACT

Background: Photodynamic therapy (PDT) is approved for treatment of actinic keratoses (AKs) and field-cancerisation. Pretreatment with pharmacological compounds holds potential to improve PDT efficacy, through direct interaction with PpIX formation or through an independent response, both of which may improve PDT treatment.
Objective: To present the currently available clinical evidence of pharmacological pretreatments prior to PDT and to associate potential clinical benefits with the pharmacological mechanisms of action of the individual compounds.
Methods: A comprehensive search on the Embase, MEDLINE, and Web of Science databases was performed.
Results: In total, 16 studies investigated 6 pretreatment compounds: 5-fluorouracil (5-FU), diclofenac, retinoids, salicylic acid, urea, and vitamin D. Two of these, 5-FU and vitamin D, robustly increased the efficacy of PDT across multiple studies, illustrated by mean increases in clearance rates of 21.88% and 12.4%, respectively. Regarding their mechanisms, 5-FU and vitamin D both increased PpIX accumulation, while 5-FU also induced a separate anticarcinogenic response. Pretreatment with diclofenac for four weeks improved the clearance rate in one study (24.9%), administration of retinoids had a significant effect in one of two studies (16.25%), while salicylic acid and urea did not lead to improved PDT efficacy. Diclofenac and retinoids demonstrated independent cytotoxic responses, whereas salicylic acid and urea acted as penetration enhancers to increase PpIX formation.
Conclusion: 5-FU and vitamin D are well-tested, promising candidates for pharmacological pretreatment prior to PDT. Both compounds affect the haem biosynthesis, providing a target for potential pretreatment candidates. Key Words: Photodynamic Therapy, Actinic Keratosis,Pre-tretment,Review,enhancement

1. Introduction

Skin cancer remains the most common malignancy diagnosed worldwide [1]. Keratinocyte carcinoma consisting of basal cell carcinoma and squamous cell carcinoma (SCC) represents the majority of these cases and is recurrently associated with premalignant actinic keratosis (AK). AKs are lesions found in areas of the skin frequently exposed to the sun with a higher tendency to occur in people with fairer skin (Fitzpatrick skin types I and II) [2]. Common locations include the face, scalp, and upper extremities often surrounded by photodamaged skin [3].

There are several treatment options available for AK lesions. The choice of therapy depends on a number of factors such as anatomical location affected, patient compliance, inconvenience related to AK lesions, cost, availability of the treatment modality, and the individual patient's risk of cancer [4]. Lesion-directed therapies involve direct treatment of individual AKs, usually through cryotherapy, curettage, or surgery [5]. These treatments are suitable for isolated lesions and may

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be effective against visible AKs. Field-directed therapies aim to target field-cancerisation where larger areas of the skin have become dysplastic. Treatments include photodynamic therapy (PDT) as well as the administration of topical agents including but not limited to 5-fluorouracil (5-FU), imiquimod, tirbanibulin, or diclofenac [6]. Field-directed therapies allow for treatment of areas with multiple or diffuse AKs lesions to address both visible and subclinical lesions.

PDT selectively targets diseased cells which allows the treatment of multiple AKs in the same session [7]. PDT requires the presence of three components: (1) a photosensitiser, (2) a light source, and (3) intracellular oxygen. The photosensitiser is a light-sensitive molecule that accumulates within the target tissue. Upon illumination with the light source, the photosensitiser becomes excited and contributes to the production of reactive oxygen species through a photochemical reaction [7,8]. The resulting species generate significant oxidative damage to macromolecules within target cells leading to the destruction of neoplastic tissue (left panel Fig. 1). The cytotoxic effect of PDT is mediated by apoptosis, autophagy, and necrosis, and is thought to be accompanied by changes to the vasculature and local inflammation [9].

Two pro-drug photosensitisers are currently in use to treat AKs: 5-aminolevulinic acid (ALA) and its methylated derivate, methyl aminolevulinate (MAL). ALA is primarily used in America in combination with blue light, whereas Europe and Australia tend to administer MAL with red light illumination [10,11]. The use of daylight and artificial, indoor daylight as the activating light source demonstrates the same clinical efficacy of conventional PDT with the benefit of reduced treatment duration and pain [12,13].

ALA occurs naturally inside the cell where it is metabolised into the endogenous photosensitiser protoporphyrin IX (PpIX) through the haem biosynthesis pathway (right panel Fig. 1) [14]. PpIX is converted into haem by the rate-limiting enzyme ferrochelatase (FECH) [15], which inhibits the endogenous production of ALA [16]. Exogenous application of the photosensitiser circumvents this feedback mechanism and allows PpIX to accumulate within the target cell ready for photoactivation [17]. Compared to normal cells, transformed cells demonstrate a distinct regulation of haem biosynthesis enzymes that further favours this accumulation of PpIX [18].

While PDT offers efficient treatment of thin and superficial AKs, the efficacy is reduced against 'difficult to treat' lesions, such as thicker AKs or lesions on acral sites. To improve the clinical outcome, combinational approaches have gained impact. Physical pretreatment through procedures such as curettage, ablative fractional laser (AFL), and microneedles is efficient at priming the skin before PDT treatment [19]. But physical pretreatment must be performed by a professional and will, therefore, increase the duration of in-clinic treatment.

Pharmacological pretreatment is the approach of combining pharmacologically active compounds with PDT. Pretreatment is typically administered by the patients themselves in the days or weeks leading up to the in-clinic PDT procedure. The aim is to enhance the susceptibility of AK lesions to the effects of PDT, potentially improving treatment outcomes.

In this review, we present the currently available clinical evidence of pharmacological pretreatments prior to photodynamic therapy of actinic keratosis. We associate potential clinical benefits with the mechanisms of action of individual compounds with the aim to provide a perspective on emerging pretreatments for PDT.

2. Methods

2.1. Outcomes

The primary outcome was to assess the clinical efficacy of pharmacological pretreatment used in combination with PDT and compare it to PDT monotherapy. Efficacy rates were evaluated based on reported clearance rates for the interventions.

The secondary outcome was to describe the mechanisms of action used by the identified pretreatment compounds to increase PDT efficacy by additional review of the literature.

2.2. Inclusion and exclusion criteria

The inclusion criteria were as follow: 1) article written in English, 2)

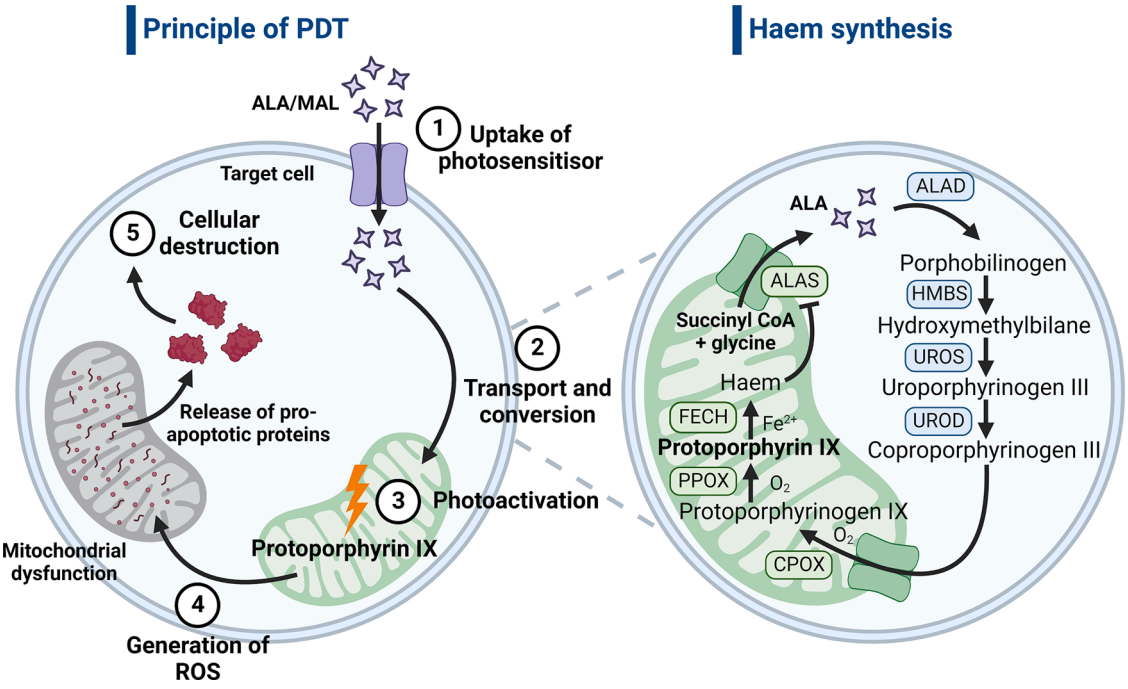

Fig. 1. Overview of photodynamic therapy (left) and the involvement of the haem biosynthesis pathway (right). Abbreviations: ALA: 5-aminolevulinic acid, ALAD: ALA dehydratase, ALAS: ALA synthase, CPOX: coproporphyrinogen oxidase, FECH: ferrochelatase, HMBS: hydroxymethylbilane synthase, PDT: photodynamic therapy, PPOX: protoporphyrinogen oxidase, ROS: reactive oxygen species, UROD: uroporphyrinogen decarboxylase, UROS: uroporphyrinogen III synthase.

data reporting on pharmacological pretreatment prior to photodynamic therapy of AK lesions, and 3) assessment of AK clearance of both pharmacological pretreatment combined with PDT and PDT monotherapy. Identified reviews were manually checked for appropriate references, and data were retrieved from the original publications. No restrictions were applied to the type of PDT, the location of the AKs, or the length of the follow-up period. Case reports, conference abstracts, uncontrolled studies, and non-human studies were excluded. Studies on physical pretreatments or pharmacological treatment administered after PDT were also not included.

2.3. Search strategy

Searches focused on pharmacological pretreatment of PDT in AK patients were conducted on the databases Embase, MEDLINE, and Web of Science on the 7th of October 2022. Free text and Mesh terms were used to ensure all relevant studies were identified. In brief, the applied search strings followed the composition of "(photodynamic therapy) and (actinic keratosis) and (pretreatment)" with specific variations and Mesh terms presented in supplementary material 1. Duplicates were eliminated with the use of Rayyan [20].

After the initial screening of titles, abstracts, and keywords, inclusion in the review was decided based on a full-text assessment performed in Rayyan [20] by two authors (CML and CP). Any potential disagreements were resolved through discussion with a third author (MH). The references of included articles were manually examined for possible inclusion.

2.4. Data extraction and summary measures

From the included articles, data were extracted by one author (CP) on the following points: 1) pretreatment compound, 2) administration of pretreatment, 3) PDT procedure, 4) study design, 5) anatomical region of the treated AKs, and 6) the efficacy of pretreatment and PDT monotherapy on AK lesion clearance.

3. Results

The database searches identified 450 references from Embase (237), MEDLINE (129), and Web of Science (84), of which 122 were removed due to duplication. A total of 328 references underwent screening of titles, abstracts, and keywords, which left 19 articles eligible for full-text assessment. Of these, a total of 16 studies were included in the review (Fig. 2).

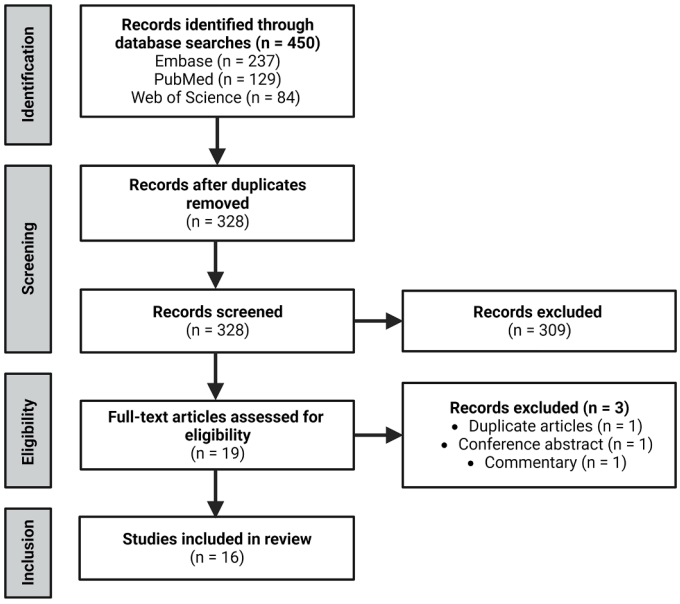

Fig. 2. Flow diagram of the record identification and selection process.

From the included studies, six compounds were reviewed: 5-FU, diclofenac, retinoids, salicylic acid, urea, and vitamin D. Table 1 provides an overview of the clinically controlled trials that considered pharmacological pretreatment prior to PDT with specific details given for study characteristics, the pretreatment regimen, photosensitisers and PDT procedure, and clearance rate.

In order to elucidate the mechanisms of pharmacological pretreatment, we have summarised the pretreatment compounds into two categories based on the effects when combined with PDT. Some compounds exhibit a direct effect on the PDT procedure by interfering with the haem biosynthesis pathway, increasing the concentration of PpIX available for photoactivation. Other compounds induce a response independent of PDT: some with a distinct anticancer effect, while others have less defined mechanisms of action. Certain compounds elicit dual actions, affecting both PpIX levels and inducing an independent response.

Among the six compounds, three had an effect on the PpIX concentration, either directly (vitamin D) or due to their properties as penetration enhancers (salicylic acid and urea), two (diclofenac and retinoids) elicited PDT-independent responses, and one (5-FU) was found to both increase PpIX accumulation and induce a separate anticancer response. Table 2 provides a summary of the mechanisms of action of these compounds as monotherapies and in combination with PDT.

3.1. Salicylic acid and urea

Salicylic acid and urea are penetration enhancers. In combination with PDT, their use should theoretically increase the uptake of the photosensitiser into the skin. In turn, the photosensitiser will be converted into PpIX through the haem biosynthesis pathway (Figs. 1 and 3). An increase in PpIX will allow for greater efficacy of PDT, potentially boosting the lesion clearance rate.

Two studies have reported on the use of salicylic acid and urea prior to PDT treatment of AK patients [21,22]. These were published between 2004 and 2016 and included 15 patients treated with salicylic acid and 31 patients treated with urea. The treatment course consisted of either a 10% salicylic acid cream applied 24 h prior to PDT, or a 40% urea cream administered 24 h prior to PDT or daily applications in the week leading up to PDT.

Gholam et al. reported no difference in lesion clearance rate between pharmacological pretreatment and curettage before PDT. In terms of tolerability, 57.3%, 42.6%, and 20% of patients pretreated with salicylic acid, urea, and curettage, respectively, interrupted the PDT procedure during light exposure because of unbearable pain [21]. The second study increased urea application to seven days prior to PDT, but no additional effect was observed [22]. While no difference in pain was reported in this study, the current evidence indicates that salicylic acid and urea should be considered inefficient, and at worst, unnecessarily harmful, pretreatments for PDT.

3.2. Vitamin D

Vitamin D (Vit D) is a steroid hormone produced in the skin in response to sun exposure. Topical application of cholecalciferol (Vit D_3) has been used in the treatment of psoriasis [23], and more recently in combination with 5-FU chemotherapy to treat AK lesions [24].

In combination with PDT, vitamin D is reported to act as a differentiating agent with a direct effect on PpIX concentration. As cancer cells are poorly differentiated, compounds can be administered to induce cellular differentiation to offset malignant transformation. Upon addition of vitamin D, expression of the differentiation marker E-cadherin and CCAAT-enhancer binding proteins (C/EBPs) [25,26], a group of nuclear transcription factors, are induced. In turn, C/EBPs induce the

Table 1
Overview of compounds and their efficacies as pharmacological pretreatments for PDT.

Compound	Administration of compound	PDT	Study design	Anatomical region	Efficacy (Clearance rate)
Salicylic acid					
Salicylic acid [21]	10% cream 24 h prior to PDT	MAL-PDT Light: Red CUR: No	Observational retrospective study Patients: 15 F-U: 1 month	Face and scalp	CUR+PDT: 68.5% Comp+PDT: 61.4%
Urea					
Urea [21]	40% cream 24 h prior to PDT	MAL-PDT Light: Red CUR: No	Observational retrospective study Patients: 14 F-U: 1 month	Face and scalp	CUR+PDT: 68.5% Comp+PDT: 60.8%
Urea [22]	40% cream daily for 1 week	ALA-PDT Light: Blue CUR: No	Clinical comparison trial Patients: 17 F-U: 5 months	Face	÷, no significant change was reported
Vitamin D					
Calcipotriol [27]	0.005% cream daily for 15 days	MAL-PDT Light: DL CUR: No	Non-randomised comparison study Patients: 11 F-U: 3 months	Face and scalp	PDT: 70% Comp+PDT: 85%*
Calcipotriol [28]	0.005% cream daily for 15 days	MAL-PDT Light: Red CUR: Yes	Randomised controlled trial Patients: 20 F-U: 12 months	Scalp	PDT: 77.5%% Comp+PDT: 90.7%*
Calcipotriol [29]	0.005% cream twice daily for 14 days	MAL-PDT Light: Red CUR: No	Randomised clinical trial Patients: 66 F-U: 12 months	Face	AFL+PDT: 79.9% Comp+AFL+PDT: 89%*
Calcitriol [30]	0.3% cream daily for 14 days	MAL-PDT Light: DL CUR: Yes	Randomised controlled trial Patients: 36 F-U: 3 months	Upper extremities	PDT: 57.9% Comp+PDT: 64%
Cholecalciferol [31]	Oral intake of 10,000 IU daily for 5 or 14 days	ALA-PDT Light: Blue CUR: No	Non-randomised cohort-controlled trial Patients: 58 F-U: 3–6 months	Face and scalp	PDT: 54.4% Comp+PDT: 72.5%*
Tacalcitol [32]	0.0004% cream daily for 15 days	ALA-PDT Light: Red CUR: Yes	Randomised comparison study Patients: 21 F-U: 3 months	Extremities	PDT: 31.5% Comp+PDT: 44.4%*
Non-steroidal anti-inflammatory drugs					
Diclofenac [37]	3% diclofenac gel twice daily for 1 month	ALA-PDT Light: Red CUR: No	Randomised controlled study Patients: 10 F-U: 12 months	Upper extremities	PDT: 64.5% Comp+PDT: 89.4%*
Retinoids					
Adapalene [41]	0.1% gel twice daily for 1 week	ALA-PDT Light: Red CUR: No	Randomised comparison study Patients: 15 F-U: 2 months	Upper extremities	PDT: 57.0% Comp+PDT: 79.0%*
Tazarotene [42]	0.1% gel twice daily for 1 week	ALA-PDT Light: Blue CUR: No	Randomised comparison study Patients: 10 F-U: 2 months	Upper extremities	PDT: 79.0% Comp+PDT: 89.5%
Chemotherapeutics					
5-fluorouracil [25]	5% cream daily for 6 days	MAL-PDT Light: Red CUR: No	Randomised clinical trial Patients: 8 F-U: 3 months	Face, scalp, and upper extremities	PDT: 30% Comp+PDT: 70%*
5- fluorouracil [48]	5% cream daily for 6 days	MAL-PDT Light: Red CUR: No	Controlled clinical trial Patients: 17 F-U: 3 months	Face, scalp, and upper extremities	PDT: 45% Comp+PDT: 75%*

(*continued on next page*)

Table 1 (*continued*)

Compound	Administration of compound	PDT	Study design	Anatomical region	Efficacy (Clearance rate)
5- fluorouracil [49]	5% cream twice daily for 1 week	ALA-PDT Light: Blue CUR: No	Randomised controlled trial Patients: 17 F-U: 12 months	No defined region	PDT: 82.6% Comp+PDT: 100%*
5- fluorouracil [50]	5% cream twice daily for 1 week	MAL-PDT Light: DL CUR: Yes	Randomised controlled trial Patients: 24 F-U: 3 months	Upper extremities	PDT: 51.8% Comp+PDT: 62.7%*
5- fluorouracil [51]	5% cream daily for 6–7 days	ALA-PDT Light: Blue CUR: No	Randomised controlled trial Patients: 30 F-U: 3 months	No defined region	Comp: 83.8% PDT: 78.9% Comp+PDT: 90%

*: signifies significant change when compared to control treatment ($p < 0.05$), ÷: signifies lack of information in the original article. Abbreviations: AFL: ablative fractional laser, ALA: 5-aminolevulinic acid, comp: (pretreatment) compound, CUR: curettage treatment prior to PDT, DL: daylight, F-U: follow-up, IU: international units, MAL: methyl aminolevulinate, PDT: photodynamic therapy.

Table 2
Overview of the mechanisms of action of the included pretreatment compounds and their effects in combination with PDT.

Compound	Mechanism of action	Effect on PDT procedure?	Proposed effect in combination with PDT
Penetration enhancers	• Enhances skin penetration	Yes	• Increases uptake of PS • Increases accumulation of PpIX • Greater effect of PDT
Vitamin D	• Induces differentiation • Reduces proliferation	Yes	• Upregulates CPOX expression • Downregulates FECH expression • Increases accumulation of PpIX • Stimulates TNF-α mediated apoptosis • Greater effect of PDT
Diclofenac	• Inhibits COX-2 activity • Reduces pro-inflammatory cytokines • Inhibits angiogenesis	No	• Inhibits COX-2-mediated survival • Stimulates TNF-α mediated apoptosis • Overall clearance boosted by PDT and diclofenac • Reduces associated inflammation
Retinoids	• Induces differentiation • Reduces proliferation	No	• Upregulates p53 expression • Induces caspase proteins • Overall clearance boosted by PDT and retinoids
5-fluorouracil	• Interferes with thymidylate synthase • Impair DNA replication • Induces apoptosis	Yes	• Upregulates CPOX expression • Downregulates FECH expression • Increases accumulation of PpIX • Greater effect of PDT • Impairs DNA replication • Overall clearance boosted by PDT and 5-FU

Abbreviations: 5-FU: 5-fluorouracil, COX-2: cyclooxygenase 2, CPOX: coproporphyrinogen oxidase, FECH: ferrochelatase, PDT: photodynamic therapy, PpIX: protoporphyrin IX, PS: photosensitiser.

upregulation of the enzyme immediately upstream of PpIX (coproporphyrinogen oxidase: CPOX), while also downregulating the expression of FECH responsible for PpIX's conversion into haem [25,26] (right panel of Fig. 1 and Fig. 3). This creates an accumulation of PpIX

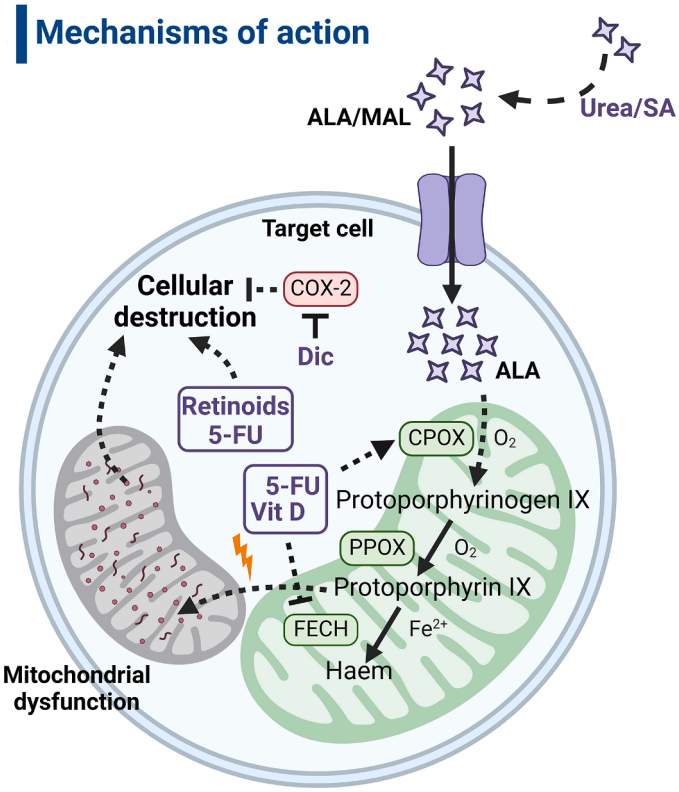

Fig. 3. Mechanisms of action of the pretreatment compounds in combination with PDT. Abbreviations: 5-FU: 5-fluorouracil, ALA: 5-aminolevulinic acid, COX-2: cyclooxygenase 2, CPOX: coproporphyrinogen oxidase, Dic: diclofenac, FECH: ferrochelatase, MAL: methyl aminolevulinate, PPOX: protoporphyrinogen oxidase, SA: salicylic acid, Vit D: Vitamin D.

within the mitochondria that will make cells more susceptible to PDT-induced destruction. The cytotoxicity following vit D-PDT treatment is reported to be mediated by TNF-α and such, the extrinsic apoptotic pathway facilitated by caspase-8 and caspase-3 [26].

Six studies on pretreatment with vit D were included: five on topical administration and one on oral delivery [27–32]. The vit D compounds consisted of either cholecalciferol, calcitriol, or the synthetic analogues calcipotriol or tacalcitol. The strength of the vit D cream varied between 0.0004% and 0.3%, and the treatment course lasted either 14 or 15 days. The dosage of oral vitamin D was 10,000 international units (IU) daily for 5 or 14 days, depending on the serum vit D level. The studies were published between 2018 and 2022 with a total population of 212 AK

patients.

Galimberti reported a lesion clearance of 85% following topical application of calcipotriol for 15 days prior to PDT compared to 70% of PDT alone [27]. The effects of calcipotriol were further corroborated by two studies. Following a 13.2% increase in lesion clearance, Torezan et al. proposed a synergistic effect when AK lesions were pretreated with vitamin D, evident by no significant increase in mean lesion count between the 3- and 12-month follow-up while PDT monotherapy increased from 2.65 to 3.2 lesions in the same period [28]. A similar increase in clearance rate was observed in AK lesions treated with vitamin D for two weeks before one session of AFL-assisted PDT (AFL+PDT: 79.9% versus vit D-AFL-PDT: 89%) [29].

Topical application of tacalcitol prior to PDT improved the clearance rate to 44.4% compared to 31.5% of PDT monotherapy [32]. Piaserico et al. reported a 15.6% increase in clearance of thicker AKs (grade II and III) when treated with calcitriol for two weeks before PDT. However, this effect was lost when all lesions were considered [30]. Oral pretreatment with 10,000 IU cholecalciferol increased lesion clearance to 72.5% compared to 54.4% of PDT alone. The treatment course was well tolerated with no additional side effects [31]. At present, the clinical data indicate a significant potential for vitamin D as a pretreatment for PDT.

3.3. Diclofenac

Compared to healthy skin, AK lesions demonstrate an increase in cyclooxygenase-2 (COX-2) expression [33], which has led to treatment with non-steroidal anti-inflammatory drugs such as diclofenac. Diclofenac functions as a COX-2 inhibitor to reduce the concentration of prostaglandins and other pro-inflammatory, angiogenic, and proliferative mediators. As a treatment for AKs, diclofenac is administered in combination with hyaluronic acid twice a day for up to 90 days [34].

Unlike the previous two compounds, diclofenac is not reported to affect PpIX. Instead, diclofenac acts independently to induce a cytotoxic response that in combination with PDT may increase the therapeutic potential. Activation of COX-2 stimulates keratinocyte survival through the induction of proliferation and apoptosis inhibition [35]. By inhibiting COX-2, diclofenac stimulates a proapoptotic environment that facilitates the elimination of diseased cells. Similar to the combination of vitamin D and PDT, this apoptotic response seems to be mediated by death ligands such as TNF-α [36].

Van der Geer and Krekels reported in 2009 a study with ten AK patients on the effects of diclofenac as pretreatment for PDT. Diclofenac was applied daily for one month prior to PDT treatment. 12 months after the procedure, the authors observed an increase in clearance rate to 89.4% for the combined therapy compared to 64.5% for PDT alone [37]. Out of ten patients, one demonstrated a severe reaction following diclofenac treatment with the group experiencing greater overall pain compared to PDT alone, but this observation was not significant.

3.4. Retinoids

Vitamin A consists of a group of fat-soluble compounds referred to as retinoids. These include naturally occurring derivates and synthetic compounds such as adapalene and tazarotene. While the use of retinoids reportedly has beneficial effects on keratinocyte proliferation and differentiation [46], their application in the treatment of AKs is limited due to variable clinical outcomes [38,39].

Retinoids' mechanism of action in combination with PDT remains unknown. Based on the available literature, we propose that retinoids do not directly affect the efficacy of PDT but instead may provide an additional, independent response. In keratinocytes, the addition of all-trans-retinoic acid upregulates p53 and induces a proapoptotic environment facilitated by the increased expression of caspase proteins related to the intrinsic apoptotic pathway [40]. This cytotoxic response induced by retinoids may further sensitize target cells to the effects of PDT.

Two studies on pretreatment with retinoids were included: one on the use of adapalene and one on tazarotene published in, respectively, 2021 and 2011 with a total of 25 patients. In both studies, a 0.1% gel was applied twice a day for one week before PDT.

Galitzer reported an improved lesion clearance following adapalene pretreatment (79%) when compared to PDT alone (57%) with no additional side effects [41]. However, administration of tazarotene prior to PDT led to no significant change in lesion clearance (PDT: 79% versus retinoid-PDT: 89.5%) [42]. While the treatment had similar tolerability to PDT monotherapy, an increase in erythema was observed in the pretreatment group. Because of these varied outcomes, more research is needed before retinoids can be considered an appropriate candidate for PDT pretreatment.

3.5. 5-fluorouracil

The use of 5-fluorouracil as a treatment for skin lesions has been a staple in dermatology since the 1960s. 5-FU is a pyrimidine nucleoside analogue that inside the cell is converted into active metabolites capable of inhibiting thymidylate synthase [43]. Without thymidylate synthase, the synthesis of thymidine is inhibited, which induces detrimental changes to other nucleotides, impairs DNA replication, and induces apoptosis [44]. 5-FU preferentially affects malignant cells and is, therefore, commonly used to treat actinic keratosis [45].

Similar to vitamin D, 5-FU acts as a differentiating agent evident by the induction of E-cadherin following 5-FU treatment. Application of 5-FU before PDT increases the accumulation of PpIX in -skin exposed to ultraviolet radiation and SCC tumours of hairless mice [46,47]. The accumulation is caused by an upregulation of CPOX immediately upstream of PpIx production, while expression of the rate-limiting FECH responsible for its conversion into haem (Fig. 1) is reduced. Accumulation of PpIX within target cells combined with the inherent cytotoxic effect of 5-FU increases the apoptotic response when 5-FU is administered prior to PDT [47].

Five studies on pretreatment with 5-FU were included. All studies used a 5% 5-FU cream, and treatment courses lasted either six or seven days. The studies were published between 2015 and 2021 with a total of 96 patients with AKs.

Maytin and Anand reported a lesion clearance of 70% following 5-FU pretreatment compared to 30% of MAL-PDT alone [25]. Two years later, the authors published another study with the same effect (PDT: 45% versus 5-FU-PDT: 75%) [48]. A similar response was observed with 5-FU pretreatment prior to both ALA-PDT (PDT: 82.6% versus 5-FU-PDT: 100%) [49] and daylight-PDT (PDT: 51.8% versus 5-FU-PDT: 62.7%) [50]. Of the five included references, one study reported no difference in lesion clearance following 5-FU pretreatment. However, a rechallenge with another cycle of 5-FU significantly reduced AK lesions in the pretreatment group [51]. Taken together, these studies indicate that pretreatment with 5-FU prior to PDT may increase the clinical outcome.

4. Discussion

In this review, we have described the effects of six pharmacological pretreatment compounds and related the observed clinical effects with their mechanisms of action. The pretreatments are diverse compounds with varying clinical efficacies. Based on the presented evidence, 5-FU and vitamin D are the most promising pretreatment candidates for PDT.

In comparison to other treatment options available for actinic keratosis, PDT has demonstrated great efficacy in treating wide areas of dysplastic tissue. Treatment duration is brief, and the side effects are mostly limited to treatment-associated pain that can be mitigated using daylight as the activating light source. The one caveat seems to be its efficacy against 'difficult to treat' lesions.

Piaserico et al. demonstrated that vit D pretreatment improved the treatment of thicker lesions. While the overall lesion clearance remained

unchanged, vit D pretreatment significantly increased lesion clearance when grade II and III lesions were considered [30]. The overall clearance rates for these lesions were 55.24% for pretreatment and 39.58% for PDT monotherapy, 57.80% and 45.22% for grade II, and 47.06% and 17.24% for grade III AKs, respectively. While Van der Geer and Krekel reported an increased lesion clearance following diclofenac pretreatment, it failed to improve lesion thickness, further emphasising the different mechanisms of action.

Considering the mean increases in lesion clearances, diclofenac ranks first with 24.9%, while vit D ranks last with 12.4%. 5-FU and retinoids demonstrate mean increases of 21.88% and 16.25%, respectively, while salicylic acid and urea both decrease the efficacy compared to the control treatment. Despite the reported efficacies, there is limited evidence regarding the use of diclofenac and retinoids in combination with PDT with only one and two studies, respectively.

We propose 5-FU and vitamin D as the most promising candidates for PDT pretreatment. Both compounds are differentiating agents that work through the regulation of the haem biosynthesis pathway to increase PpIX accumulation, and both have demonstrated additional effects when administered prior to PDT [25,47].

The mechanism provided for 5-FU is very similar to the effects reported following the combination of oral methotrexate (MTX) and PDT. MTX enhances PpIX accumulation and increases both selectivity and efficacy of PDT [52,53]. As MTX treatment has been associated with hepatotoxicity [54], 5-FU emerged as a promising substitute because of similarities between MTX's and 5-FU's interference with the pyrimidine biosynthesis pathway [55]. In addition to this anticancer effect [56] and its potentiation of PDT's efficacy, 5-FU pretreatment was recently shown to enhance anti-tumor immunity through increased induction of both the innate and adaptive immune responses [57].

The mechanisms of the other four compounds in combination with PDT are less defined. The descriptions of how diclofenac and the retinoids may act are deduced through studies unrelated to PDT [36,40] and therefore describe a general response rather than a mechanism specific to the photochemical reaction of PDT. While salicylic acid and urea are presented as penetration enhancers with an effect on PpIX formation due to an increased photosensitiser uptake, there is no such report in terms of PpIX fluorescence or PDT efficacy. It is, therefore, possible that the potential to stimulate a cytotoxic response does not necessarily result in a boosted effect when combined with PDT.

From this review and previous publications [58], it is clear that pretreatment with pharmacological compounds prior to PDT significantly improves the clinical outcome in patients with actinic keratosis. Given the current evidence, differentiating agents such as 5-FU and vitamin D demonstrate the greatest potential to increase lesion clearance rates, but more trials may be needed to further specify pretreatment regimes.

CRediT authorship contribution statement

Celina Pihl: Writing – review & editing, Writing – original draft, Visualization, Investigation, Data curation. **Catharina M. Lerche:** Writing – review & editing, Supervision, Investigation, Data curation. **Flemming Andersen:** Writing – review & editing, Funding acquisition. **Peter Bjerring:** Writing – review & editing, Funding acquisition. **Merete Haedersdal:** Conceptualization, Data curation, Project administration, Writing – review & editing.

Declaration of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.pdpdt.2023.103703.

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