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A combination of photodynamic therapy and antimicrobial compounds to treat skin and mucosal infections: a systematic review†

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Background: Antimicrobial photodynamic therapy (aPDT) is a growing approach to treat skin and mucosal infections. Despite its effectiveness, investigators have explored whether aPDT can be further combined with antibiotics and antifungal drugs. *Objective*: To systematically assess the *in vivo* studies on the effectiveness of combinations of aPTD plus antimicrobials in the treatment of cutaneous and mucosal infections. *Materials and methods*: Searches were performed in four databases (PubMed, EMBASE, Cochrane library databases, ClinicaTrials.gov) until July 2018. The pooled information was evaluated according to the PRISMA guidelines. *Results*: 11 full-text articles were finally evaluated and included. The best aPDT combinations involved 5-aminolevulinic acid or phenothiazinium dye-based aPDT. In general, the combination shows benefits such as reducing treatment times, lowering drug dosages, decreasing drug toxicity, improving patient compliance and diminishing the risk of developing resistance. The mechanism of action may be that first aPDT damages the microbial cell wall or membrane, which allows better penetration of the antimicrobial drug. *Limitations*: The number of studies was low, the protocols used were heterogeneous, and there was a lack of clinical trials. *Conclusions*: The additive or synergistic effect of aPDT combined with antimicrobials could be promising to manage skin and mucosal infections, helping to overcome the microbial drug resistance.

Introduction

The problem of microbial drug resistance

After more than half a century of decline, microbial infections are now increasing again (not decreasing) with a significant impact on mortality and morbidity rates, as well as the associated financial burden. This renewed increase is largely caused by the development of multidrug resistance (MDR).[1] Microbial resistance to antibiotics in both community and hospital settings has been increasing worldwide in the last two decades, and seems likely to continue to increase further in the near future.[2,3]

New molecules are being developed to meet the need for compounds with activity against resistant pathogens.[4] In particular, the Infectious Diseases Society of America has supported an initiative to develop ten new antibacterial agents by the year 2020: "10 × 20 Initiative".[5] However, despite these new antibiotics, the increasing prevalence of antibiotic-resistant bacterial infections has not been halted.[6] To meet this threat, alternative non-antibiotic therapies are necessary. Antimicrobial photodynamic therapy (aPDT) has been proposed as one alternative treatment for localized infections, especially cutaneous or mucosal infections.[7,8]

Antimicrobial photodynamic therapy: achievements and challenges

aPDT is based on the use of non-toxic dyes or photosensitizer molecules (PS) that are activated by harmless visible light in

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†Electronic supplementary information (ESI) available: Fig. 1 Simplified diagram of the photodynamic reaction. e^-: electron; H_2O_2: hydrogen peroxide; hv: photons (light); $^{\cdot}O_2^-$: superoxide anion; $^1O_2$: singlet oxygen; $^3O_2$: molecular oxygen; $^{\cdot}OH$: hydroxyl radical; PS: photosensitizer in basal state; $^1PS^*$: photosensitizer in its singlet state; $^3PS^*$: photosensitizer in excited triplet state; ROS: reactive oxygen species. See DOI: 10.1039/c8pp00534f

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the presence of oxygen; this combination is able to generate reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide or hydroxyl radical (Type I) and/or singlet oxygen (Type II). All these different ROS can oxidize various biological molecules, such as proteins, nucleic acids, and lipids, leading to cell death and destruction of microorganisms.[8,9] Fig. 1 of the ESI† summarizes the process.

One advantage of aPDT for infections is the possibility of eliminating microorganisms independently of their antimicrobial resistance pattern, and without requiring a precise microbial diagnosis. The advantages also include a broad spectrum of activity, a very rapid response time (seconds or minutes), a low probability of adverse side effects, and the modest cost of treatment.[10] Whereas the most important limitations are the possibility of regrowth of those microorgnisms that were not inactivated during the irradiation, some phototoxicity can occur also in some tissues or host cells, pain during the irradiation with some protocols, and the lack of standardized clinical protocols.[8,11,12]

An option that paves the way for the future is the combination of aPDT treatment with conventional antimicrobials in order to achieve an additive or synergistic therapeutic effect or even to overcome antimicrobial resistance.[13,14] This original approach points to potentially new and versatile applications for the therapy of superficial cutaneous infections. This option could help widen the use of aPDT, and reduce the amount of antibiotics used, thereby diminishing the problem of MDR.[8,15] Table 1 summarizes the possible advantages. The key issue is that the addition of antimicrobials to *in vivo* aPDT might prevent microbial regrowth when the light is turned off, and the antimicrobial effects of the photogenerated ROS rapidly cease. During the preparation of the present review, another excellent review by Wozniak and Grinholc appeared, which contained some overlapping material with the present review.[14] Nevertheless, we believe that the two review articles are complementary in nature rather than duplicative.

Objective

The aim of this review is to determine the utility of the combinations of aPTD plus antimicrobials (aPDT and antimicrobial simultaneously given, aPDT followed by antimicrobial or *vice versa*) to treat skin and mucosal infections in humans or animals.

Table 1 Most important possible advantages of the combination of aPDT and classical antimicrobial treatment

1	They are complementary treatments because on using low doses of both, they obtain better results	
	aPDT:	Antimicrobial:
	Less staining of the skin	Lower dose
	Less photodynamic dose	Less side effects
	Less number of treatments	
2	No selection of resistant microorganisms	
3	Less risk of microorganism proliferation and treatment failure	

aPDT: antimicrobial photodynamic therapy.

The questions that are intended to be answered are: (1) which skin and mucous infections have been treated with combinations of aPDT and antimicrobials; (2) which methodologies have been used; and (3) what do the results indicate.

Methods

This review was written following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[16,17] The systematic review of the literature was carried out as detailed below.

Eligibility criteria

We have taken into account *in vivo* studies that used antimicrobial treatments plus aPDT against skin and mucosal infections. The specific requirements for inclusion of the studies were (1) *in vivo* studies in humans or animals including those that used animals as a model; (2) aimed to treat skin and/or mucosal infections; (3) caused by identified bacteria, yeast or fungi; (4) used antibiotics or antifungals as a fundamental part of the treatment; (5) used aPDT as a fundamental part of the treatment; (6) published in indexed journals and written in English or Spanish.

Information sources and search

Pubmed, Embase, Clinicaltrial.gov and Cochrane library databases were used. Two independent reviewers performed the search and cross-checked their findings. No time limits were used in the search for articles. The last search was carried out in July 2018. The keywords used for the search were: photodynamic therapy, PDT, antimicrobial photodynamic therapy, aPDT, photodynamic antimicrobial chemotherapy, PACT, photoinactivation, photodynamic inactivation, PDI, combination, combined treatment, antimicrobials, antibiotics and antifungals.

Study selection

All studies that meet the selection criteria were included.

Data collection process

The methodology of the antimicrobial treatment and aPDT were listed in a table. The data recapitulated in clusters were: (1) causative agent of skin and/or mucosal infection; (2) type of *in vivo* study: animal model or patients; (3) antimicrobial methodology: antibiotics or antifungals used and their application and dose; (4) aPDT methodology: PS used, parameters of irradiation (source type, wavelength and intensity), number of sessions and fluence and (5) observed effect of combined treatment on infection.

Risk of bias in individual studies

The risk of bias of individual studies was assessed in each study and taken into account at the outcome level when the data synthesis was carried out. We identified domains of bias such as selection bias, performance bias, detection bias, attri-

tion bias, reporting bias and other potential sources of bias following the recommendations of The Cochrane for the evaluation.[18]

Summary measures and the synthesis of results

A table with the collected data was created to facilitate data handling and the combination of the results of the studies. Due to the heterogeneity of the studies in terms of methodology and treatment protocol, the outcomes were presented in a descriptive manner. The observed effect of the combined treatments on infections was collected in any of the ways reported in each study (difference in proportions between groups, confidence intervals, clinical follow-up of the lesions and microbiological diagnosis).

Risk of bias across studies

The risk of bias across studies such as publication bias and selective reporting was assessed in order to appraise the accumulated evidence.

Results

Study selection

A huge number of papers contained the keywords selected for our search. Nevertheless after applying the eligibility criteria, the number was drastically reduced to a total of 11 studies, which have been assessed and included in this review.

Study characteristics & synthesis of results

The 11 studies that fulfilled the selection criteria were screened: the group was composed of clinical cases ($N = 7$, ten patients) or experiments in animal models using mice ($N = 2$) or *Galleria mellonella* larvae ($N = 2$). Among them, 2 dealt with bacterial infections caused by Gram negatives, 2 against atypical mycobacterial infections, 2 against candidiasis, and 5 against dermatophytoses and other mold infections. All were assessed and included in the review, grouped depending on the causal agent of skin and/or mucous infection. Table 2 summarizes the data extracted from the studies reporting combinations of *in vivo* aPDT plus other treatments against bacterial infections, candidiasis, atypical *Mycobacterium* species, dermatophytoses and mold infections of the skin and mucosa.

Risk of bias within studies

Table 3 summarizes the risk of bias within studies. All clinical cases involved a high risk of selection, performance and detection biases because they were not randomized and there was no blinding. The clinician established the treatment protocol for the specific patient, the patient agreed with it and therefore the evaluators of the result (patient and clinical staff) knew the applied treatment. There was only one patient treated with systemic antibiotics for a cutaneous infection caused by *Mycobacterium fortuitum* on the hands who received additionally two sessions of ALA-PDT in only one hand which showed a significant improvement compared with the other hand.[24] This is the reason why in this study, the risk of detection bias was considered unclear instead of high. Attrition bias risk was considered unclear in all cases, because the loss of patients was not reported, but it is not known whether other patients refused the treatment or did not complete it. In the clinical cases (one patient per study except in that of Sun *et al.* with four patients), these are not clinical trials with a significant number of patients.[19] Reporting bias risk also was considered unclear: the study protocols are available but the possible results are not prewritten and the results are reported descriptively. Only in the study of Gilaberte *et al.* was the clinical improvement confirmed with microbiological analysis[32] and therefore the risk was considered minor.

In the four studies using animal models, a low risk of selection bias was considered because they used a random method to establish each group. Namely, until the time of assignment, the group in which a particular animal would be included was unknown. However, a high risk of performance bias and in the reporting of the results was considered because there was no blinding of personnel either during the experiment or at the moment of evaluating the outcomes. The personnel knew the treatment for each group all the time and it cannot be excluded that this influenced the evaluation of the results. The validity of the variables with regard to the assessment of the results of the study was considered to have a low risk of bias in the two studies of Chibebe *et al.* and in the study of Baltazar *et al.* because all variables were covered by the different groups of animals.[20,21] However, not enough information was available to make a clear judgment in the study of Lu *et al.* (*e.g.*, no group of mice was only exposed to light).[22] On the other hand, the low risk of attrition and reporting biases were considered in all studies because they did not report the loss of animals in any group (no incomplete outcome data) and the protocol is available and all results are described as planned (no selective outcome reporting) with the exception of the study of Lu *et al.* in which unclear reporting bias risk was considered. This assessment is a result of not showing the data of the group treated only with the photosensitizer BF6 in the dark, although the study indicated that there was a small reduction in the bacterial luminescence from mouse wounds.

No conflicts of interest were detected in any of the eleven studies included in the review.

Results of the individual studies

Gram-negative bacteria

Pseudomonas aeruginosa is an opportunistic human pathogen especially causing infections in chronic ulcers and burns. An assay in a mouse model of wounds infected with a highly virulent *P. aeruginosa* strain combined tricationic fullerene-mediated aPDT with a suboptimal dose of tobramycin (Table 2), reporting a synergistic therapeutic effect capable of curing 60% of mice who would otherwise all die with this fatal infection.[22] These results were in agreement with those presented by Collins *et al.* in a study against biofilm-forming

Table 2 Studies of combinations of *in vivo* aPDT plus other treatments used against bacterial infections, candidiasis, atypical *Mycobacterium* species and dermatophytes and mold infections of the skin and mucosa

Type of infections	Subject	Strain	Antibiotic/ antifungal	Antibiotic/ antifungal dose, administration	PS	Light source	Wavelength (nm)	Power (mW cm^{-2})	Fluence (J cm^{-2}) and aPDT sessions	Treatment groups/type	Synergy	Observed effect	Bibliography
5 × 5 mm excisional wound down to the panniculus carnosus + 50 μl of a PBS suspension containing 2.5×10^7 CFU	Murine model, 36 male Balb/c	*P. aeruginosa* ATCC 19660	Tobramycin	Intraperitoneal injection: 6 mg kg^{-1} for 1 day (modest regimen)	BF6	Non-coherent white lamp	400–700	200	180 (1 session 15 min after the infection)	No treatment; aPDT; tobramycin; aPDT + tobramycin	Yes	60% survival of mice *vs.* 20% with tobramycin alone	Lu *et al.* (2010)[22]
10 μl PBS inoculum aliquots into the hemocoel *via* the last left proleg containing $>10^7$ CFU per larva	*G. mellonella* larvae, 17 per group	*E. faecium* clinical isolates & 2158; *E. faecalis* clinical isolates & OG1RF; (some VRE)	Ampicillin, streptomycin, gentamicin or vancomycin	Hemocoel injections 120 min after the infection: ampicillin 150 mg kg^{-1}, streptomycin 15 mg kg^{-1}, gentamicin 6 mg kg^{-1} and vancomycin 50 mg kg^{-1}	MB	Non-coherent lamp band-pass filter	660 ± 15	ND	0.9 (1 session 90 min after the infection)	Nothing; inoculated with PBS; antibiotics; MB-aPDT; MB; light	Yes	Higher sensitivity to these antibiotics	Chibebe *et al.* (2013a)[20]
Multiple skin abscesses in the hands	Patient	*M. fortuitum*	Clarithromycin, rifampin, levofloxacin, and ethambutol hydrochloride tablets	Antibiotics for 1 month.	5-ALA	Semiconductor laser optical fiber or LED	635 or 633	84	100 (2 sessions in 10 days)	Not improved after 2 weeks of antibiotics alone; aPDT was applied in left hand; right hand self-control	Yes	Cure < *t*: left hand improved much faster than right hand	Gong *et al.* (2016)[24]
Skin infections	4 patients	*M. fortuitum*	Clarithromycin, moxifloxacin hydrochloride, amikacin, rifampicin, ethambutol hydrochloride, levofloxacin	Different treatment regimens	5-ALA	LED	633	84	100 (every 10 days for a total of 3–5 sessions)	aPDT + antibiotics at the same *t*	Yes	Cure	Sun *et al.* (2017)[19]
		M. chelonae subsp. *abscessus*	Clarithromycin, moxifloxacin hydrochloride, amikacin, imipenem cilastatin sodium										
		M. gordonae	Clarithromycin, moxifloxacin hydrochloride, amikacin, sulfamethoxazole										
		M. gilvum	Clarithromycin, moxifloxacin hydrochloride										
Cutaneous granuloma	Patient	*C. albicans*	Itraconazole	Itraconazole for 1 month	5-ALA	ND	ND	ND	2 sessions	aPDT + traconazole at the same *t*	Yes	Clinical cure	Cai *et al.* (2018)[26]

Table 2 *(Contd.)*

Type of infections	Subject	Strain	Antibiotic/ antifungal	Antibiotic/ antifungal dose, administration	PS	Light source	Wavelength (nm)	Power (mW cm^{-2})	Fluence (J cm^{-2}) and aPDT sessions	Treatment groups/type	Synergy	Observed effect	Bibliography
5 μl PBS inoculum aliquots into the hemocoel *via* the last left proleg containing $>10^6$ CFU per larva	*G. mellonella* larvae, 17 per group	Fluconazole-resistant *C. albicans* Can37	Fluconazole	Hemocoel injection: 14 mg kg^{-1} before or after the exposure to light	MB	Non-coherent lamp band-pass filter	660 ± 15	ND	0.9 (1 session)	Nothing; inoculated with PBS; fluconazole; MB-aPDT; MB; light	Yes	Higher survival	Chibebe *et al.* (2013b)[21]
Dermatophytosis, 1×10^6 conidia per animal	Murine model, six C57BL/6	*T. rubrum* ATCC 28189	Ciclopirox olamine	0.65 mg per mice topically every 48 h over a period of 7 days	TBO	LED	630	ND	42 (daily)	Uninfected; infected without treatment; infected with treatments	Yes	Lesions improved	Baltazar *et al.* (2015)[30]
Fixed cutaneous sporotrichosis on left arm	Patient	*S. schenckii*	Itraconazole	Intermittent low 200 mg day^{-1}, 7 days, 1 per month doses	MB	LED	639.8 ± 10	19	37 (3 sessions every 2 weeks)	Itraconazol and oral terbinafine or topical MAL-aPDT alone was used without success. Intralesional MB-aPDT + itraconazole was applied	Yes	Microbiological and clinical cure	Gilaberte *et al.* (2014)[32]
Refractory chromoblastomycosis on the right ankle	Patient	*F. monophora*	Terbinafine	250 mg day^{-1} oral	5-ALA	LED	635	36.8	2 (9 sessions weekly)	Not improved after >1 year with antifungal drugs; aPDT + terbinafine at the same *t*	Yes	Lesions improved clinically and no recurrence	Hu *et al.*, (2015)[33]
Refractory chromoblastomycosis	Patient	*F. monophora*	Terbinafine	250 mg day^{-1} oral	5-ALA	ND	ND	ND	5 sessions weekly (2 periods)	Not improved after 2 years with antifungal drugs; aPDT + terbinafine at the same *t*	Yes	Lesions improved clinically, no mycologic or complete clinical cure	Yang *et al.*, (2012)[34]
Chromoblastomycosis	Patient	*A. alternata*	Itraconazole	Short course of itraconazole (400 mg day^{-1} oral for 15 weeks) and subsequent aPDT	5-ALA	LED	633 ± 10	80	2	Itraconazole and subsequent 5-ALA-aPDT	Yes	Clinical cure	Liu and Xia (2014)[37]

5-ALA: 5-aminolevulinic acid; aPDT: antimicrobial photodynamic therapy; BF6: BF6 fullerene; CFU: colony forming unit; ICG: indocyanine green; LED: light-emitting diode; MB: methylene blue; ND: no data; TBO: toluidine blue O; PBS: phosphate-buffered saline; PS: photosensitizer; *t*: time; VRE: *Enterococcus* vancomycin resistant.

Table 3 Risk of bias within studies. ✓: Low risk of bias; ✗: high risk of bias; ?: unclear bias risk (there is not enough information available to make a clear judgment); CC: clinical case; AM: animal model murine; AG: animal model using G. mellonella larvae

		Bias						
Bibliography	Type of study	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment, personnel-reported (detection bias)	Blinding of outcome assessment, all-cause (detection bias)	Incomplete outcome data (attrition bias)	Selective outcome reporting (reporting bias)
Lu *et al.* (2010)[22]	AM	✓	✓	✗	✗	?	✓	?
Chibebe *et al.* (2013a)[20]	AG	✓	✓	✗	✗	✓	✓	✓
Gong *et al.* (2016)[24]	CC	✗	✗	✗	✗	?	?	?
Sun *et al.* (2017)[19]	CC	✗	✗	✗	✗	✗	?	?
Cai *et al.* (2018)[26]	CC	✗	✗	✗	✗	✗	?	?
Chibebe *et al.* (2013b)[21]	AG	✓	✓	✗	✗	✓	✓	✓
Baltazar *et al.* (2015)[30]	AM	✓	✓	✗	✗	✓	✓	✓
Gilaberte *et al.* (2015)	CC	✗	✗	✗	✗	✗	?	✓
Hu *et al.* (2015)[33]	CC	✗	✗	✗	✗	✗	?	?
Yang *et al.* (2012)[34]	CC	✗	✗	✗	✗	✗	?	?
Liu and Xia (2014)[37]	CC	✗	✗	✗	✗	✗	?	?

P. aeruginosa: using the same antibiotic plus aPDT, although based on another PS (*meso*-tetra (*N*-methyl-4-pyridyl) porphine tetra tosylate), they observed greater inactivation and a decrease in tobramycin MIC.[23]

Enterococcus faecium has emerged as one of the most important pathogens in healthcare-associated infections worldwide due to its intrinsic and acquired resistance to many antibiotics, including vancomycin.[6,20] *Enterococcus faecalis* is an opportunistic pathogen isolated from patients with different types of infections including wounds and surgical-sites.[20]

Methylene blue(MB)-aPDT combined with antimicrobial agents (ampicillin, streptomycin, gentamicin or vancomycin) increased the sensitivity of bacteria to these antibiotics.[20] The survival of *G. mellonella* larvae infected with a vancomycin-resistant *Enterococcus* (VRE) strain, was extended when vancomycin was administered after aPDT (Table 2). However, when vancomycin or aPDT was administered separately, no extension of caterpillar survival was observed. It is possible that the permeabilization of the bacterial cell wall by the sub-lethal aPDT makes it more susceptible to the antibiotic. The results with *E. faecium* and *E. faecalis* were similar.

Atypical mycobacteria

Mycobacterial skin infections other than *Mycobacterium tuberculosis* and *Mycobacterium leprae* are a type of refractory infection typically treated with different combinations of various antibiotics over 6–12 months.[19]

Mycobacterium fortuitum is highly resistant to primary antituberculosis drugs, and thus is very difficult to treat. A patient with multiple skin abscesses caused by *M. fortuitum* was treated with different antibiotics (clarithromycin, rifampin, levofloxacin, and ethambutol hydrochloride) plus application of a protocol of 5-aminolevulinic acid (5-ALA)-aPDT (Table 2). The combination significantly shortened the treatment time for the infection.[24]

The efficacy and safety of 5-ALA-PDT combined with different antibiotics were tested in four patients diagnosed with atypical mycobacterial skin infections caused by *M. fortuitum*, *Mycobacterium chelonae* ssp *abcessus*, *Mycobacterium gordonae* or *Mycobacterium gilvum* respectively. The four patients were treated for a total of 3 months and displayed no signs of recurrence over 3 months of follow-up. Due to the fact that each different atypical *Mycobacterium* species is sensitive to different drugs, the combination of antibiotics used to treat these infections was different in each case, but all included clarithromycin and moxifloxacin hydrochloride[19] (Table 2).

Yeasts

Candida albicans is the most prevalent pathogenic yeast. It does not only cause skin infections, but also oral and genital mucosal infections.[25]

Cai *et al.* presented a clinical case of a cutaneous granuloma caused by *C. albicans* treated with itraconazole for 1 month and two sessions of 5-ALA-aPDT (Table 2). The patient who had suffered the infection for two years was cured

and the authors concluded that including aPDT in the treatment was beneficial.[26]

Chibebe *et al.* confirmed that MB-aPDT prolonged the survival of *G. mellonella* larvae infected with *C. albicans*. A fluconazole-resistant *C. albicans* strain was used to test the combination of MB-aPDT and fluconazole (Table 2). Administration of fluconazole either before or after exposing the larvae to aPDT significantly prolonged the survival of the caterpillars compared to each treatment used alone.[21] These results were in agreement with those presented by Giroldo *et al.* and Lyon *et al. in vitro*. The former demonstrated that both planktonic suspensions and biofilms were much more susceptible to antifungal drug treatments after MB-aPDT, which may be due to an increase in membrane permeability by the aPDT.[27] They later evaluated *in vitro* the combination of MB-aPDT and fluconazole against fluconazole-resistant *C. albicans* strains, and reported a synergistic effect.[28]

Dermatophytes and non-dermatophyte fungi

Trichophyton rubrum is an anthropophilic fungus that colonizes the upper layers of dead skin causing athlete's foot, onychomycosis and ringworm throughout the world.[29]

To our knowledge, the study from Baltazar *et al.* is the only one that explores the combination of cyclopiroxolamine, a hydroxypyridone antifungal drug, and toluidine blue O-aPDT against *T. rubrum* in a murine model (Table 2). aPDT alone significantly reduced the fungal burden by 87% compared with the untreated group and it was 64% more efficient than cyclopiroxolamine alone, and both treatments together showed a synergistic combination, reducing the damage caused by the fungus in the skin. aPDT also reduced the myeloperoxidase levels, but not the activity of N-acetylglucosaminidase, suggesting that there was a reduction in neutrophils but not of macrophages within the affected tissue. Furthermore, this study correlated the effective production of ROS with PDT efficacy.[30]

Sporothrix schenckii causes a subcutaneous mycosis known as sporotrichosis. Infection generally occurs by traumatic inoculation of soil, plants, and organic matter contaminated with the fungus into the skin.[31]

Gilaberte *et al.* used intralesional 1% MB-aPDT in combination with intermittent low doses of itraconazole in a patient with recalcitrant cutaneous sporotrichosis (Table 2). Complete microbiological and clinical responses were obtained when both treatments were combined, in contrast to the antifungal treatment alone, which could not be fully administered to the patient due to a pre-existing chronic liver disease. However, MB-aPDT alone was not clinically tested, and it could be the case that the entire effect was due to the aPDT alone. In fact *in vitro* testing with the strain isolated from the patient showed that whereas MAL-aPDT was not able to photoinactivate the fungus, any of the phenothiazinium dyes tested (including MB) produced more than 6 $\log_{10}$ reduction in the number of CFU mL^{-1}.[32]

Fonsecaea spp. is the main causative agent of chromoblastomycosis, one of the most frequently encountered mycoses in tropical and temperate regions, and which is associated with low rates of cure and high relapse rates.[33,34]

There are two reports of refractory cases of this infection successfully being treated with a combination of 5-ALA-PDT plus terbinafine[33] or 5-ALA-PDT plus itraconazole[34] (Table 2). The latter report was supported by an *in vitro* study that showed growth inhibition of 5-ALA-aPDT against *Fonsecaea monophora.* No response was obtained at first with terbinafine (250 mg day^{-1} oral, 6 months) and itraconazole (200 mg day^{-1} oral, 1 month) alone or with these two antifungals in combination (2 periods of 1 month) and then 5-ALA-aPDT was added (Table 2). As a result, the lesions improved but new lesions developed after the cessation of PDT. Thereafter, positive clinical improvement was obtained when voriconazole (200 mg day^{-1} oral) was combined with terbinafine (250 mg day^{-1} oral) for 2 months.

The results obtained in these studies[33,34] agree with previous reports[35,36] that concluded that aPDT could be successfully employed in combination with systemic antifungal drugs, and which proposed itraconazole plus aPDT as the combination with the greatest potential benefit in the treatment of *F. monophora* infections although they did not specify a protocol.

Alternaria alternata is a rare etiologic agent of phaeohyphomycosis in immunocompromised patients, and which had never been reported to cause chromoblastomycosis until the clinical case presented by Liu and Xia. They described *A. alternata* as the etiological agent of chromoblastomycosis for the first time, and the patient was successfully treated with a short course of itraconazole and subsequent 5-ALA-aPDT. The usual management strategy consists of long courses of antifungal chemotherapy, such as itraconazole or terbinafine which is continued until there is clinical resolution, which is usually after several months of therapy. When PDT with 20% 5-ALA cream incubated for 3 hours followed by irradiation was tested, the lesions clinically improved after the first two sessions[37] (Table 2).

Risk of bias across studies

The small number of studies (N = 11) that make up our entire group of analysis, the fact that most of them were clinical cases with heterogeneous treatment protocols (N = 7) and none were clinical trials, together constitute the main limitations of our review. These limitations mean that the accumulated evidence was reduced and not free from bias: the risk of bias within studies has already been commented on section Risk of bias within studies and we must add the risk of publication bias that we cannot quantify. Consequently, the risk of bias for each given outcome across studies is high.

Summary of evidence and limitations

In general, the combination of aPTD plus antimicrobial therapy has the potential to reduce treatment times, lower the drug dosage, avoid drug toxicity, improve patient compliance, and diminish the risk of developing resistance. Negative

Table 4 Summary of the best combined aPDT therapies for cutaneous and mucosal infections

• aPDT seems to enhance the effect of aminoglycoside antibiotics against infections caused by Gram-negative bacteria.
• The combination of antibiotics like clarithromycin or moxifloxacin hydrochloride with 5-ALA-aPDT reduces the treatment time and dose of antibiotics for atypical mycobacterial infections.
• For *Candida* spp., MB-aPDT plus oral fluconazole is the best option to overcome the resistance of *C. albicans* to this antifungal drug.
• 5-ALA and phenothiazinium dye-based aPDT are the options with most clinical evidence to be combined with cyclopiroxolamine, itraconazole or terbinafine for superficial fungal infections

effects are not reported in any clinical case or animal study analyzed. It seems that the best option is to administer the antibiotic or the antifungal drug after aPDT rather than before, although the specific mechanism of action is not completely understood. The hypothesis is that aPDT damages the microbial cell wall or membrane, which allows better penetration of the drug. On the other hand, in those infections that require a long course of antibiotics or antifungals, it seems that the repetition of the aPDT could enhance the effect of the antimicrobials (see Table 2). According to this review, there are not enough evidence to establish the best protocol for aPDT combined with antimicrobials for the different cutaneous and mucosal infections. Therefore, the length of the antimicrobial and the number of PDT sessions should be determined depending on the clinical and microbiological response. More clinical studies are needed in order to determine the optimal combinations and the best treatment protocols supported by existing evidence (Table 4).

Conclusions

aPTD combined with antimicrobial agents is promising for the management of skin and mucous membrane infections because:

(1) aPDT may increase the antimicrobial effect of antibiotics and antifungals;

(2) The combination of aPDT with conventional antimicrobials can reduce the dose needed to achieve a bactericidal/fungicidal effect;

(3) The combination may turn a microorganism that is initially resistant to a specific antimicrobial drug into a microorganism that is sensitive to that drug;

(4) In some cases, the addition of aPDT can shorten the antimicrobial treatment course.

The best option would be either to apply aPDT followed by the antimicrobial compounds or to administer periodic sessions of aPDT in long treatments with antimicrobials.

Conflicts of interest

Dr Hamblin is on the following Scientific Advisory Boards: Transdermal Cap Inc, Cleveland, OH; BeWell Global Inc, Wan Chai, Hong Kong; Hologenix Inc. Santa Monica, CA; LumiThera Inc, Poulsbo, WA; Vielight, Toronto, Canada; Bright Photomedicine, Sao Paulo, Brazil; Quantum Dynamics LLC, Cambridge, MA; Global Photon Inc, Bee Cave, TX; Medical Coherence, Boston MA; NeuroThera, Newark DE; JOOVV Inc, Minneapolis-St. Paul MN; AIRx Medical, Pleasanton CA; FIR Industries, Inc. Ramsey, NJ; UVLRx Therapeutics, Oldsmar, FL; Ultralux UV Inc, Lansing MI; Illumiheal & Petthera, Shoreline, WA; MB Lasertherapy, Houston, TX; ARRC LED, San Clemente, CA; Varuna Biomedical Corp. Incline Village, NV; Niraxx Light Therapeutics, Inc, Boston, MA. Dr Hamblin has been a consultant for: Lexington Int, Boca Raton, FL; USHIO Corp, Japan; Merck KGaA, Darmstadt, Germany; Philips Electronics Nederland B.V.; Johnson & Johnson Inc, Philadelphia, PA; Sanofi-Aventis Deutschland GmbH, Frankfurt am Main, Germany. Dr Hamblin is a stockholder in: Global Photon Inc, Bee Cave, TX; Mitonix, Newark, DE. All other authors report that they do not have any commercial or any other association that might pose a conflict of interest.

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