Antimicrobial Peptides for Bacterial Control

Subjects: Agriculture, Dairy & Animal Science

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Antimicrobial resistance to conventional drugs has resulted in high global rates of recurrent invasive infections, facilitating disease progression and reducing the likelihood of effective treatments.

antimicrobial peptides photochemotherapy

1. Introduction

In 2020, the World Health Organization warned about the appearance of strains increasingly resistant and difficult to control. The indiscriminate use of antimicrobial drugs is facilitated by inadequate medical prescriptions and substandard medications [1].

Considering the challenges related to antimicrobial resistance, other strategies for controlling infections have been suggested [2][3][4][5]. Antimicrobial photodynamic therapy (aPDT) has been used to inactivate microorganisms and treat infections [2][3][4][5]. aPDT involves the application of a photosensitizing agent (PS), an LED source corresponding to the absorption band of the PS, and the presence of oxygen. This therapy has several advantages in the treatment of infections from microorganisms, such as the wide spectrum of action and a low mutagenic potential in exposed cells [5].

When comparing aPDT with other therapies, it has the advantage of local PS application, restricting the treatment to the area of interest, thus preventing systemic side effects. There is also an immediate onset of action and elimination of virulence factors secreted by resistant microorganisms [6]. Lastly, the literature did not report the development of bacteria and fungi resistance to aPDT [3][7].

Studies have shown that microbial biofilms reduce the susceptibility to aPDT compared to planktonic cultures [3]. Considering the protection endowed by the extracellular matrix (ECM), it is difficult for the PS to penetrate the deeper layers of the microbial biofilm, impairing aPDT activity [8]. To overcome this limitation, aPDT associated with enzymes or antifungal agents was more effective for microbial inactivation than aPDT alone [4][8]. Additionally, antimicrobial peptides (AMP) have been used alone [9][10], combined with aPDT [11][12], or by conjugating a PS to the AMP molecule [13][14][15][16][17][18][19][20][21][22][23][24][25][26][27][28][29][30], presenting satisfactory results in pathogenic microorganism inactivation.

AMP are molecules expressed by all living organisms and responsible for the innate defense system against pathogen infection, including viruses, bacteria, fungi, and parasites [31]. AMP are oligopeptides with up to 50 amino

acids with a broad spectrum of action against microorganisms [32][33]. This new class of compounds has boosted science for new methodologies for synthesizing, isolating, purifying, analyzing, and quantifying peptides [34]. The presence of cationic residues (Arg and Lys) in AMP promotes a positive liquid charge for this structure, resulting in the interaction with the negative cell membrane of the target organism, such as bacteria [34]. Another important aspect of the construction of the AMP amphipathic structure is the high fraction of hydrophobic amino acids (>50%) [35], which is vital for membrane penetration. The biological activity of AMP is closely related to their structure, and these could be classified as α-helix, β-sheet, extended peptides, and both α-helix and β-sheet peptides [36], with the first two appearing more frequently [37]. Although the molecular target of some peptides is inside the cell, as non-membrane disruptive AMP [38], most peptides interact with the anionic components of the membranes of microorganisms and damage this structure [31].

The literature has described the association of AMP and aPDT to explore the best properties of both treatments, increasing the effectiveness and decreasing the time of application [11][12]. AMP can form pores in cell membranes and present biofilm activity [10], which leads to the penetration of the PS into the membrane, facilitating the inactivation of structures through LED photoexcitation [11]. Other advantages of association treatments are reduced effective dose, minimized toxicity potential, and reduced treatment costs [11][39].

2. Synthesis of Results

The results of the systematic review show that all articles had an in vitro experimental design and 3 of them were both in vitro and in vivo experimental studies [27][29][30]. Moreover, of the 20 articles analyzed, 18 performed the therapy with a portion of the PS redirected to AMP and only 2 studies performed the therapy combined with AMP [11][12]. The shortest and longest irradiation times were 30 s [13] and 20 h [20][21], respectively. The most commonly used PS were chlorin e6 [11][12][23][27][29][30] and porphyrin [13][15][16][18][20][21][24][25]. Additionally, the most frequently used microorganism in the assay was *Staphylococcus aureus* [11][13][14][16][17][18][19][20][21][23][25][26][28][29][30], followed by *Escherichia coli* [11][13][15][16][17][18][19][20][21][25][26][29][30]. Most of the studies analyzed evaluated the microorganisms in suspension (planktonic culture) and only 4 evaluated the therapy in a biofilm culture [11][23][27][30] (**Table 1**).

Table 1. Summary of the characteristics of the studies included.

Study (Year)	Study Design	Peptide	Irradiation Time	Wavelength	Photosensitizer	Microorganism	Culture Type	Sample Size	Outcomes
Bourré et al. 2010 [13]	In vitro	Tat	30, 43, 60, and 120 s	410 nm	Tetracks (phenol) and porphyrin	*Escherichia coli* *Staphylococcus aureus* *Pseudomonas aeruginosa* *Streptococcus pyogenes*	Suspension	ND	Reduction in the concentration of 1 uM from 3 to 6 log_{10} CFU/mL. The greatest effect was in the first 30 s.

Study (Year)	Study Design	Peptide	Irradiation Time	Wavelength	Photosensitizer	Microorganism	Culture Type	Sample Size	Outcomes
Yang et al. 2011 [14]	In vitro	WLBU2	100 s	652 nm	Temoporfin + WLBU2	*S. aureus* (methicillin resistant) *P. aeruginosa*	Suspension	3	Reduction by 100% for *S. aureus* (aPDT only and aPDT + peptide) and reduction by 2 log_{10} CFU/mL for *P. aeruginosa* (aPDT + peptide).
Liu et al. 2012 [15]	In vitro	WI13WF (YVLWKRKRKFCFI-amide)	2, 5, and 10 min	400 to 900 nm	Protoporphyrin IX	*E. coli* *Salmonella enteric* *Klebsiella pneumoniae*	Suspension	ND	Peptide and PS conjugate 99% lethal.
Dosseli et al. 2013 [16]	In vitro	Apidaecin	ND	600–750 nm 390–460 nm	Porphyrin	*E. coli* *S. aureus*	Suspension	ND	Reduction by 100% for *E. coli.*
Johnson et al. 2013 [17]	In vitro	$(KLAKLAK)_2$	30 min	525 nm	$(KLAKLAK)_2$ + Eosin Y	*Acinetobacter baumannii* *P. aeruginosa* *E. coli* *S. aureus* *Staphylococcus epidermidis*	Suspension	ND	Reduction by 99% for all microorganisms.
Dosseli et al. 2014 [18]	In vitro	Magainin Buforin	ND	390–460 nm	Porphyrin	*E. coli* *S. aureus* (methicillin resistant)	Suspension	ND	Reduction by 100% for all microorganisms.
Johnson et al. 2014 [19]	In vitro	$(KLAKLAK)_2$	2 min 5 min 30 min	525 nm	$(KLAKLAK)_2$ + Eosin Y	*E. coli* *S. aureus*	Suspension	3	Reduction by 50% for all microorganisms (2 min of irradiation). Reduction by 90% (5 min of irradiation). Reduction by 99.99% (30 min of irradiation).
Le guern et al. 2017 [20]	In vitro	Polymyxin B	20 h	420 nm	Porphyrin	*S. aureus* *E. coli*	Suspension	ND	Antibactericidal activity of the

Study (Year)	Study Design	Peptide	Irradiation Time	Wavelength	Photosensitizer	Microorganism	Culture Type	Sample Size	Outcomes
						P. aeruginosa			PS and peptide association on 3 strains.
De Freitas et al. 2018 [11]	In vitro	Aurein 1.2 (AU)	ND	660 nm	Methylene blue Chlorin e6	*S. aureus* *A. baumannii* *E. coli* *Enterococcus faecium*	Suspension	9	*S. aureus* reduction - MB ~ 1.0 log_{10} CFU/mL - MB + AU ~ 6.0 log_{10} CFU/mL - Ce6 and Ce6 + Au = total reduction *A. baumannii* reduction - MB ~ 1.0 log_{10} CFU/mL - MB + AU ~ 6.0 log_{10} CFU/mL - Ce6 and Ce6 + AU no significant results *E. coli* reduction - MB ~ 4.0 log_{10} CFU/mL - MB + AU ~ 4.0 log_{10} CFU/mL - Ce6 and Ce6 + AU no significant results

Study (Year)	Study Design	Peptide	Irradiation Time	Wavelength	Photosensitizer	Microorganism	Culture Type	Sample Size	Outcomes
									E. faecium reduction - MB ~ 1.0 log_{10} CFU/mL - MB + AU ~ 3.0 log_{10} CFU/mL - Ce6 ~ 1.0 log_{10} CFU/mL - Ce6 + AU = total reduction
Le guern et al. 2018 [21]	In vitro	Polymyxin B modified by lysine	20 h	420 nm	Porphyrin	*S. aureus* *E. coli* *P. aeruginosa*	Suspension	ND	Reduced antibacterial activity of polymyxin modified by lysine.
Nakonieczana et al. 2018 [22]	In vitro	CAMEL Pexiganan	668 s 1335 s 2668 s	514 nm	Rose-bengal (RB)	*P. aeruginosa*	Suspension	3	Reduction by 2.06 log_{10} CFU/mL for RB + CAM. Reduction by 6.00 log_{10} CFU/mL for RB + PEX.
Gao et al. 2019 [23]	In vitro	Magainin I	2 min 4 min 8 min	660 nm	Magainin I + Chlorin e6	*P. aeruginosa* *S. aureus* (methicillin resistant)	Biofilm	ND	*P. aeruginosa* 2 min (0.385 log_{10} CFU/mL reduction) 4 min (1.645 log_{10} CFU/mL reduction) 8 min (6.724 log_{10} CFU/mL reduction) *S. aureus* 2 min (0.922 log_{10} CFU/mL reduction)

ND: not documented; s: seconds; min: minutes: h: hour; PS: photosensitizer; ~: approximately; MB: methylene blue; RB: rose-bengal; Ce6: chlorin e6.

3. Risk of Bias Assessments for In Vitro Studies

The criteria from the OHAT Rob tool were applied to all articles included in the systematic review. The most frequent biases regarded blinding procedures. Moreover, the problem with internal validity was the lack of methodological details in the statical analyses and the performance of treatments only in microorganism suspensions (**Table 2**).

Study (Year)	Study Design	Peptide	Irradiation Time	Wavelength	Photosensitizer	Microorganism	Culture Type	Sample Size	Outcomes
									4 min (3.796 log_{10} CFU/mL

Studies/Questions	Was the Dose or Exposure Level Administered Adequately Randomized?	Was the Allocation to Study Groups Adequately Concealed?	Were the Experimental Conditions Identical Across Study Groups?	Were Research Personnel Blind to the Study Group During the Study?	Were the Outcome Data Complete without Attrition or Exclusion from the Analysis?	Is the Exposure Characterization Reliable?	Is the Outcome Assessment (Including Blinding of Assessors) Reliable?	Were There No Other Potential Threats to Internal Validity?
Bourré et al. 2010 [13]	++	++	++	--	++	++	--	--
Yang et al. 2011 [14]	++	++	++	--	++	++	--	--
Liu et al. 2012 [15]	++	++	++	--	++	++	--	--
Dosseli et al. 2013 [16]	++	++	++	--	--	++	--	--
Johnson et al. 2013 [17]	++	++	++	--	++	++	--	--
Dosseli et al. 2014 [18]	++	++	++	--	++	++	--	--
Johnson et al. 2014 [19]	++	++	++	--	++	++	--	--
Le Guern et al. 2017 [20]	++	++	++	--	++	++	--	--
De Freitas et al. 2018 [11]	++	++	++	--	++	++	--	--
Le Guern et al. 2018 [21]	++	++	++	--	++	++	--	--
Nakonieczana et al. 2018 [22]	++	++	++	--	++	++	--	--
Gao et al. 2019 [23]	++	++	++	--	++	++	--	--
De Freitas et al. 2019 [12]	++	++	++	--	++	++	--	--

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Fesse et al. 2019 [24]	++	++	++	--	++	++	--	--
Zhang et al. 2019 [25]	++	++	++	--	++	++	--	--
Chu et al. 2021 [26]	++	++	++	--	++	++	--	--
Gao et al. 2021 [27]	++	++	++	--	++	++	--	--
Judzewitsch et al. 2021 [28]	++	++	++	--	++	++	--	--
Qiu et al. 2021a [29]	++	++	++	--	++	++	--	--
Qiu et al. 2021b [30]	++	++	++	--	++	++	--	--

[12][14][22]

viability)
20 min (~42.5% viability)
30 min (~10% viability)

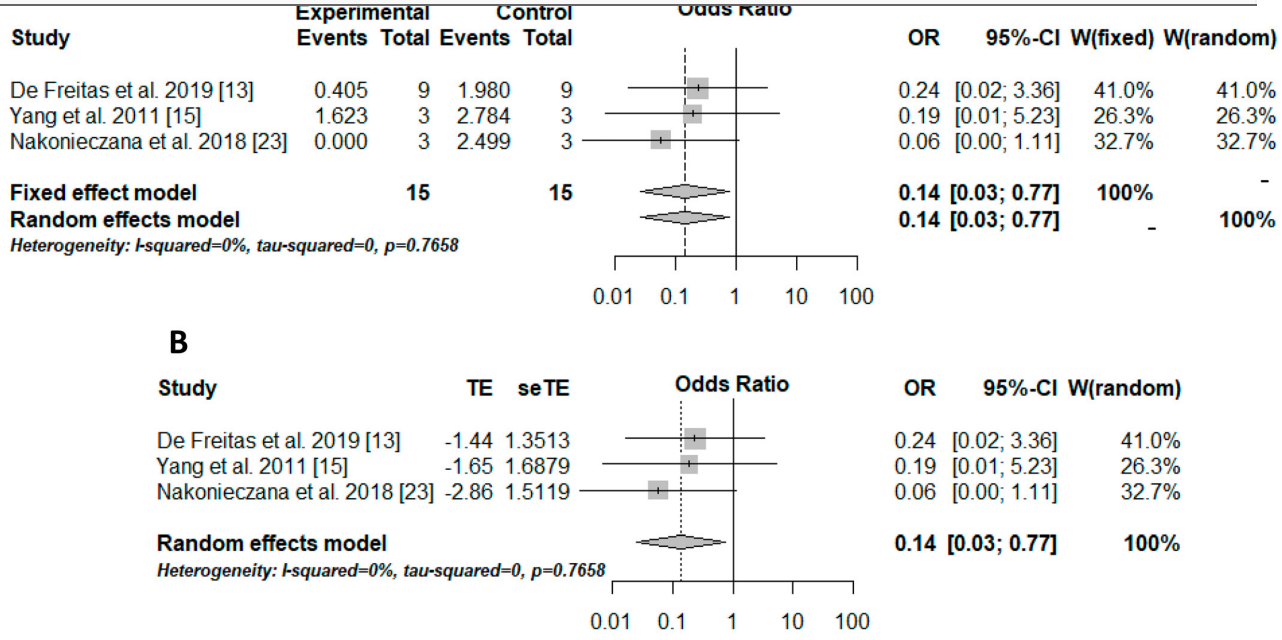

Figure 1. Illustration of the results of the quantitative analysis. The experimental group (positive events) included microorganisms that received the association therapy (aPDT + AMP), while the control group included microorganisms that received only aPDT. (**A**) results of the meta-analysis illustrated in a forest plot. OR: odds ratio; CI: confidence interval; W: weight, [12][14][22]. (**B**) trim-and-fill method results illustrated in a forest plot. TE: estimated mean; seTE: estimated standard deviation; OR: odds ratio; CI: confidence interval; W: weight, [12][14][22].