Photodiagnosis and Photodynamic Therapy 21 (2018) 211–216

Contents lists available at ScienceDirect

Photodiagnosis and Photodynamic Therapy

journal homepage: www.elsevier.com/locate/pdpdt

Antimicrobial photodynamic activity of Rose Bengal, alone or in combination with Gentamicin, against planktonic and biofilm *Staphylococcus aureus*

Vanesa Pérez-Laguna[a,b,*,1], Isabel García-Luque[c,1], Sofía Ballesta[c], Luna Pérez-Artiaga[b], Verónica Lampaya-Pérez[b], Sofía Samper[a,d,e], Pilar Soria-Lozano[b], Antonio Rezusta[a,b,f,2], Yolanda Gilaberte[a,g,2]

[a] IIS Aragón, Zaragoza, Spain
[b] Department of Microbiology, Hospital Universitario Miguel Servet, Zaragoza, Spain
[c] Department of Microbiology, School of Medicine, University of Sevilla, Sevilla, Spain
[d] Instituto Aragonés de Ciencias de la Salud, Hospital Universitario Miguel Servet, Zaragoza, Spain
[e] CIBER Enfermedades Respiratorias (CIBERES), Instituto de Salud Carlos III, Madrid, Spain
[f] Department of Microbiology, Preventive Medicine and Public Health, University of Zaragoza, Zaragoza, Spain
[g] Department of Dermatology, Hospital Universitario Miguel Servet, Zaragoza, Spain

ARTICLE INFO

Keywords:
S. aureus
Photoinactivation
Antimicrobial photodynamic therapy
Antibiotic
Biofilm

ABSTRACT

Antimicrobial photodynamic therapy (aPDT) could constitute an alternative therapy to antibiotics especially against superficial infections caused by bacteria involved in multidrug resistance processes.

The aim of this study is to compare the efficacy of aPDT using the photosensitizer rose bengal (RB), combined or uncombined with gentamicin (GN), against *Staphylococcus aureus*.

Different concentrations of RB (ranging from 0.03 to 64 μg/ml) were added to *S. aureus* in water suspensions or forming biofilms in the absence or presence of GN (1–40 μg/ml) and the samples were irradiated (18 or 37 J/cm^2). The number of viable bacteria was quantified by counting colony-forming units.

RB-aPDT shows significant photoactivity. The combination of GN and RB-aPDT exerts a synergistic bactericidal effect against planktonic *S. aureus*. On the other hand, a synergistic effect is observed only when the maximum concentration tested of RB and GN was used in biofilm.

According to these result the use of RB-aPDT alone or in combination with GN could be implemented against *S. aureus*.

1. Introduction

Microbial resistance to antibiotics in both community and hospital settings is increasing worldwide and seems likely to increase in the near future [1,2]. The microorganisms which are the leading cause of nosocomial infections, mainly involved in the resistance process (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacteriaceae*), are called the ESKAPE pathogens. They are the main targets in clinical microbiology [1,3].

S. aureus has become a major cause of community and hospital acquired infection associated with surgical wounds and with indwelling medical devices. It causes skin infections, localized abscesses, and deep-seated infections (osteomyelitis, endocarditis). On the other hand, can cause food poisoning and toxic shock syndrome [4]. *S. aureus* poses serious risks to patients with immunological diseases by the emergence of methicillin-resistant *S. aureus* (MRSA) strains through all over the world [5]. In addition, staphylococci have non-specific mechanisms of resistance, of which biofilm formation is undoubtedly the most important [6]. In biofilm state, the bacteria are attached to a substratum, interface, or to each other and are embedded in a matrix of extracellular polymeric substance which isolates and protects them. As a result, biofilm-associated infections lead to chronic diseases because the host immune response is largely

* Corresponding Author at: Department of Microbiology, Hospital Universitario Miguel Servet, Calle Padre Arrupe, Zaragoza, 50009, Spain.
E-mail address: 565686@unizar.es (V. Pérez-Laguna).
[1] These authors contributed equally to this work.
[2] These authors contributed equally to this work.

https://doi.org/10.1016/j.pdpdt.2017.11.012
Received 10 October 2017; Received in revised form 16 November 2017; Accepted 27 November 2017
Available online 28 November 2017

Table 1
Range of minimum photosensitizer concentrations (μg/ml) required to reduce *S. aureus* growth by 6 $\log_{10}$. GN, gentamicin; LED, light-emitting diode; ND: no data; RB, rose bengal; WMH, white metal halide.

		Water suspension			Biofilm
		RB μg/ml	RB+GN 1 μg/ml	RB+GN 10 μg/ml	RB+GN 40 μg/ml
515 nm LED-lamp	Fluence 18 J/cm^2	0.62	0.16	0.03	64
	Fluence 37 J/cm^2	0.31	0.07	0.03	64
WMH-lamp	Fluence 37 J/cm^2	0.31	0.07	0.03	ND

ineffective and are difficult to treat with antibiotics [6,7].

In this context, of increase of antimicrobial resistance and shortage of new effective antibiotics, alternative therapies are necessary [1,3,8]. Antimicrobial photodynamic therapy (aPDT) has been proposed as an alternative treatment for localized superficial infections due to the mechanism that requires oxygen and light [9,10]. aPDT is based on the use of non-toxic dyes or photosensitizer molecules that are activated by visible light in the presence of oxygen; this combination is able to generate reactive oxygen species (ROS) (type 1) or/and singlet oxygen ($^1O_2$) (type 2) which can oxidize many biological molecules leading to kill a target microorganism [11]. The main advantage of aPDT is the possibility of eliminating microorganisms independently of their antimicrobial resistance pattern. It is also effective against microorganisms in the biofilm state [10,12]. Moreover, the advantages include the low probability of the occurrence of side effects and the convenient cost of treatment [9]. Rose bengal (RB) is an inexpensive xanthene dye that has been used as photosensitizer in aPDT, demonstrating the efficacy of this approach in inactivating *S. aureus* [13,14].

Future directions of aPDT include the combination with common antimicrobials in order to achieve an additive or synergistic therapeutic effect or even to overcome the resistances. This original approach points to new and wide applications in the treatment of superficial skin infections. The combination of aPDT and conventional antibiotics to treat staphylococcal infections has already shown significant potential [13,15–17].

Gentamicin (GN) is an aminoglycoside antibiotic that can be administered intravenously, intramuscular or topically to treat several types of bacterial infections including those caused by *S aureus* [18]. GN has been previously shown to increase the efficacy of aPDT [15].

The aim of this study was to compare the efficacy of aPDT using the photosensitizer RB (RB-aPDT), combined or uncombined with the antibiotic GN, against *S. aureus* in water suspensions and forming biofilm.

2. Materials and methods

2.1. Chemicals and media

- Solvent: Distilled water.
- Culture Media: Columbia Blood agar (BA) (Oxoid®; Madrid, Spain) and tryptic soy broth (TSB) (Becton-Dickinson; Madrid, Spain)
- Antibiotic: Gentamicin (GN) (Sigma-Aldrich®; Madrid, Spain). For studies of bacteria in suspension, antibiotic was applied at concentrations of 1 μg/ml and 10 μg/ml, and for staphylococcal biofilms, 10, 20 and 40 μg/ml of GN were assayed.
- Photosensitizer: Rose bengal (RB), (Sigma-Aldrich-Fluka®; Madrid, Spain). Stock RB solutions were prepared and diluted in distilled water immediately prior to use. All solutions were prepared and handled under light-restricted conditions. Concentrations ranged from 0.03 to 64 μg/ml were used in both suspension and biofilm assays.

2.2. Strain

S. aureus ATCC 29213 was acquired from the American Type Culture Collection (ATCC, Rockville, MD, USA).

2.3. Light sources

Green light-emitting diode (LED) emitting at 515 ± 10 nm (power density 5.8 mW/cm^2) and white metal halide (WMH) emitting at 420–700 nm (90 mW/cm^2) lamps were used [13,19].

RB-aPDT was performed using LED lamp with fluences of 18 J/cm^2 and 37 J/cm^2 and WMH lamp at a fluence of 37 J/cm^2.

2.4. In vitro photodynamic treatment of planktonic bacteria

The procedure used was adapted from our previous work as follows [13]. Bacteria seeded on BA were cultured aerobically overnight at 35 °C. The inoculum was prepared in distilled water and adjusted to 0.5 ± 0.03 on the McFarland scale (concentrations in the range of > 10^7 colony-forming units per ml [CFU/ml]) and was deposited into 96-well microtiter plates. Varying concentrations of the RB (ranged from 0.03 to 2.5 μg/ml) were added, in the presence or absence of GN (1 μg/ml or 10 μg/ml). RB concentrations were chosen based on published results from previous experiments performed in our laboratory using 2-fold serial dilutions from 640 to 0.03 μg/ml of RB [13]. The final volume in each well was 100 μl. Irradiation proceeded with no preincubation period; the suspensions were immediately subjected to irradiation with fluences of either 18 J/cm^2 or 37 J/cm^2 using green LED lamp and 37 J/cm^2 using the WMH lamp. Control samples were subjected to identical treatment, in the absence or presence of the photosensitizer, and were either kept in darkness or irradiated to