PALGO JOURNAL OF MEDICINE AND MEDICAL SCIENCE
ISSN 2476-8340
Volume 5 Issue 4, August 2018.Page 60 - 65
http://www.palgojournals.org/PJMMS/Index.htm
Corresponding Authors Email:sunningning520@163.com

THE ANTIMICROBIAL PHOTODTNAMIC ACTIVITIES AGAINST THE PATHOGENIC MICROORGANISMS WITH SNN-1.

Ning-Ning Sun[1], Xiang-Yan Min[1], Phuong Anh Nguyen Thi[1], Ying-Hua Gao[1], Yi-Jia Yan[2,3], Zhi-Long Chen[1*]

Department of pharmaceutical Science& Technology, College of Chemistry and Biology, Donghua University, Shanghai 201620, China[1]
Shanghai Xianhui Pharmaceutical Co.Ltd., Shanghai 200433, China[2]
Ningbo Dongmi Pharmaceutical Co., Ltd, Ningbo 315899, Zhejiang, P.R. China[3]

Accepted 30 August, 2018

Antimicrobial photodynamic therapy represents an alternative method of killing drug-resistant pathogens. Efforts have been made to develop delivery systems for hydrophobic drugs to improve the photokilling. This study is to evaluate the photodynamic effect of photosensitizer SNN-1 on Escherichia coli and Staphylococcus aureus. After the pre-incubation period, the drug was washed out and then was performed with laser light irradiation (690nm±15nm). Negative control samples were not exposed to SNN-1 or light. For the suspension, colonies were counted with (colony-forming units per milliliter (CFU/mL)). The efficiency was dependent on the photosensitizer concentration and light dose. The photodynamic therapy of SNN-1 caused photokilling of the both stains of E. coli and S. aureus. The live bacterial colony count represents that the photosensitizer SNN-1 to both strains reduced by 80% and 85% respectively. This therapy may represent an alternative treatment for eradicating planktonic strains.

Keywords: Antimicrobial photodynamic therapy; Pathogenic microorganisms; Escherichia coli; Staphylococcus aureus;

INTRODUCTION

Bacterial infections play a central role in focal infections and systemic infections which were the leading cause of morbidity and mortality. Antibiotics were discovered in the late 1930s which rescue millions of infected patients. However, overuse of antibiotics has contributed to antibiotic-resistant bacteria which is called super-bacterial. Thus, patients with infections caused by drug-resistant bacteria are at increased risk of worse clinical outcomes and death. In this basic project, we choose Gram-negative bacteria E. coli and Gram-positive bacteria S. aureus as our study object. Escherichia coli and Staphylococcus aureus are the most common pathogens causing healthcare-associated infections such as hospital-acquired infections. This topic aim was to study the use of photodynamic therapy with the commonly used clinical photosensitizer MB and anti-tumor photosensitizer SNN-1.

The antimicrobial photodynamic therapy (aPDT) has emerged as an alternative treatment of skin and mucosa lesions and infection (Wainwright M 1998). However, unlike antitumor PDT,(Zolfaghari, Parjam S 2009) which has been used in some countries as an established therapeutic option, aPDT is still in the experimental phase. Photodynamic therapy produces no drug-resistance and is safe without side-effects. (Bredell MG, Besic E, Maake C, Walt H 2010). With light excitation, the PS undergoes a transition to a more energetic state in which it is capable to react with molecules present in the medium and transfer energy oxygen species (ROS; type I reaction), (WM Gallagher, LT Allen, C O'Shea 2005) or transfer energy to molecular oxygen, generating the highly reactive singlet oxygen (type II reaction) (Konopka K, Goslinski T 2007). The products of these reactions cause lethal oxidative damage to the bacterial cells.

Methylene blue is an alkaline dye that has a positive charge in water. Methylene blue (MB) as a photosensitizer has been used for the treatment of cyanide and nitrite poisoning, methemoglobinemia, periodontitis, dental caries, etc. because of its safety, reliability and non-toxicity. (Wainwright M. 2003). Kathleen Fernandes Grego used snake as an animal model and reported that photodynamic therapy can be used to treat infectious stomatitis. They isolated the infected tissues of infectious stomatitis and identified the main infectious strains as Pseudomonas aeruginosa and Escherichia coli. The snake was treated with methylene blue(MB) as a photosensitizer. (O'Connor AE, Mc Gee MM, Likar Y 2011) After the treatment, it was found that the snake infection site had been replaced by the normal flora.(Kathleen FernandesGrego.2017).

SNN-1 is BF_2-chelated tetraaryl-azadipyrromethenes which is non-porphyrin photosensitizers for the development of novel PDT agents. Development of such agents may identify compounds with improved efficacy, reduced side-effects

and increased possibilities for modification.
The aim of this study was to evaluate the photodynamic effect of SNN-1 on the E. coli and S. aureus in vitro.

MATERIALS AND METHODS

All solvents and reagents were obtained from commercial suppliers and used without further purification unless otherwise stated. Two photosensitizer solutions of different concentrations (15μM, 25μM, 50μM) were prepared in PBS, and PDT treatment excitation light wavelengths of photosensitizers were selected according to the absorption spectrum. Irradiation of PS was carried out using a light system composed of laser light irradiation with the lamps uniformly distributed into the device in order to provide a uniform irradiation of the plate. The laser device provided a maximum emission at 690nm and the irradiance delivered was 50mW/cm^2. The light doses used were 15, 25, or 50J/cm^2, resulting in approximately 5, 8, 16 min of irradiation time, depending on the bacterial strain.

In vitro experiments

Bacterial culture and culture conditions

The bacterial strains used in this study were obtained from the Type Culture Collection of the Chinese Academy of Sciences. Both isolates were maintained in Luria-Bertani-LB (Tryptone, Yeast Extract, NaCl) medium and Agar in solid medium. For the experiments, these bacteria were individually inoculated in 5mL of LB and grown aerobically overnight at 37. Each culture was harvested after centrifugation at 2000rpm for 10min, washed twice with sterile distilled water, and resuspended in PBS. Cell suspensions were standardized by spectrophotometer calibrated at 600nm wavelength to give final concentration of 1 x 10^7 cells mL^{-1}.

Photodynamic inactivation studies in planktonic suspensions

Aliquots of 150uL of the E. coli and S. aureus suspensions were individually transferred to separate Eppendorf and centrifuged to remove the supernatant. The cells were incubated with different concentrations (15μM, 25μM, 50μM,) MB and SNN-1 for 30min in the dark. After this pre-incubation time, PBS was added to the cells. The samples were transferred to a 12-well plate that was placed on the laser light device for illumination at light 15, 25, 50J/cm^2 for the E. coli and S. aureus. Controls were established in order to determine whether MB and SNN-1 alone (dark toxicity controls) or light alone (light control-LC) induced any effect on bacteria viability. Therefore, additional wells containing E. coli and S. aureus suspensions were exposed to the drug under identical conditions to those described above, but not to laser irradiation, and others were exposed only to irradiation. A negative control (NC) was also established consisting of bacterial suspensions not exposed to MB and SNN-1or laser.

The viability of the planktonic suspensions was evaluated by the number of colony-forming units per milliliter (CFU/mL). Aliquots of the contents of each well were serially diluted tenfold in sterile saline to give dilution of 10^{-1} to 10^{-3} times the original concentration. Triplicate 25uL aliquots were plated onto agar plates. All plates were aerobically incubated at 37 for 24h and thereafter colony counts of each plate were quantified (CFU/mL) using a digital colony counter (CP 600 plus; Phoenix Dentsply Ind.e.Com. Equipamentos Cientificos Ltda., Araraquara, SP, Brazil). For analytical purposes, CFU/mL values were transformed into logarithm ($\log_{10}$). For the transformation into $\log_{10}$, the number counted and the dilution used were placed in a formula (CFU/mL=number of colonies x 10^n/q), in which the “n” represents the absolute number of the dilution (0, 1, 2, 3, or 4), and “q” represents the amount (mL) used for each dilution when plated (0.025 mL).

RESULTS AND DISSUSSION

Effect of aPDT on colony counts of planktonic suspensions

The photodynamic antimicrobial effect of MB and SNN-1 against E. coli (CFU/mL) is presented in **Fig.1a**. The E. coli bacterial growth was directly dependent on the light fluence (15, 25, 50J/cm^2). MB and SNN-1 promoted photokilling of E. coli when irradiated with the light fluence of 15J/cm^2. When combined with the light fluence of 15J/cm^2, MB and SNN-1 caused 1.5 and 2 $\log_{10}$ reduction of bacterial cell survival, respectively. The LC-control and 0J didn’t cause a significant decrease of E. coli bacterial growth and only the combined use of PS and light was capable of reducing the number of colonies.

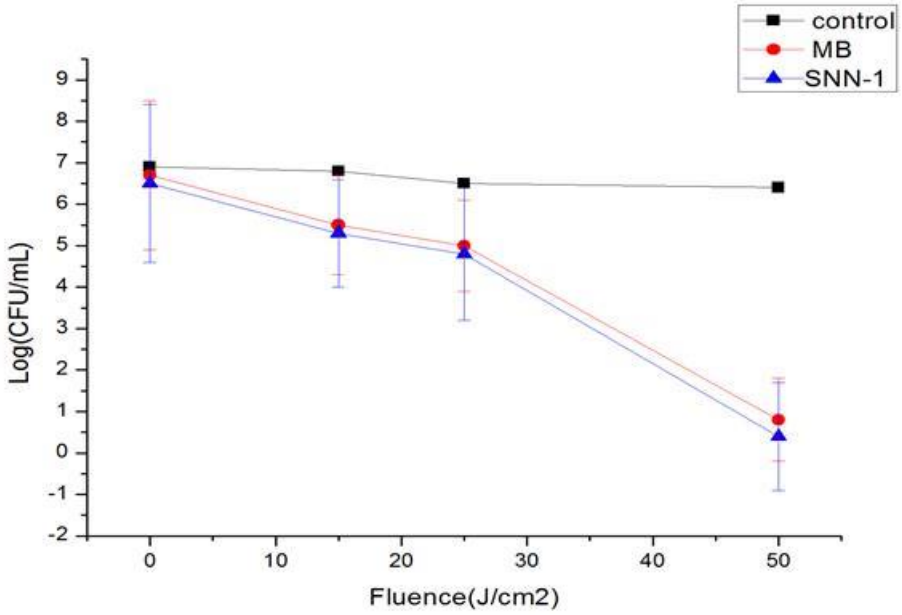

Fig.1a Line-chart graphic representation of mean values and standard deviation of logarithmic of survival counts (CFU/mL) of E. coli planktonic suspensions according to the light fluence (0, 15, 25, 50J/cm^2).

The photodynamic antimicrobial effect of MB and SNN-1 against S. aureus (CFU/mL) is presented in **Fig.1b**. The S. aureus bacterial growth was directly dependent on the light fluence (15, 25, 50J/cm^2). MB and SNN-1 promoted photokilling of S. aureus when irradiated with the light fluence of 15J/cm^2. When combined with the light fluence of 15J/cm^2, MB and SNN-1 caused 2 and 2.5 $\log_{10}$ reduction of bacterial cell survival, respectively. The LC-control and 0J didn't cause a significant decrease of S. aureus bacterial growth and only the combination of PS and light was capable of reducing the number of colonies.

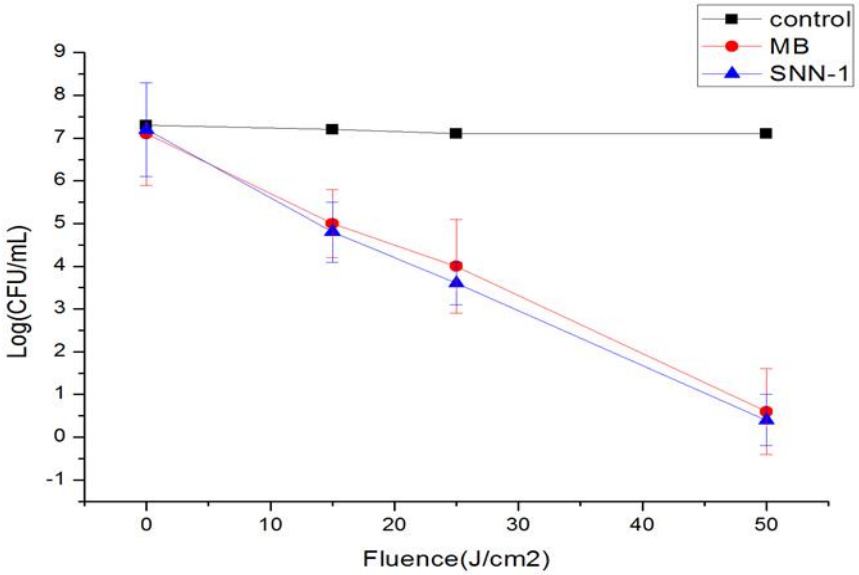

Fig.1b Line-chart graphic representation of mean values and standard deviation of logarithmic of survival counts (CFU/mL) of S. aureus planktonic suspensions according to the light fluence (0, 15, 25, 50J/cm^2).

The photodynamic antimicrobial effect of MB and SNN-1against E. coli (Bacterial survival) is presented in **Fig.2a**. The E. coli bacterial growth was directly dependent on the light fluence (15, 25, 50J/cm^2). MB and SNN-1 promoted photokilling of E. coli when irradiated with the light fluence of 50J/cm^2. When combined with the light fluence of 50J/cm^2, MB and SNN-1caused 80% and 85% reduction of bacterial cell survival, respectively. The LC-control and 0J didn't cause a significant decrease of E. coli bacterial growth and only the combination of PS and light caused the reduction of the survival.

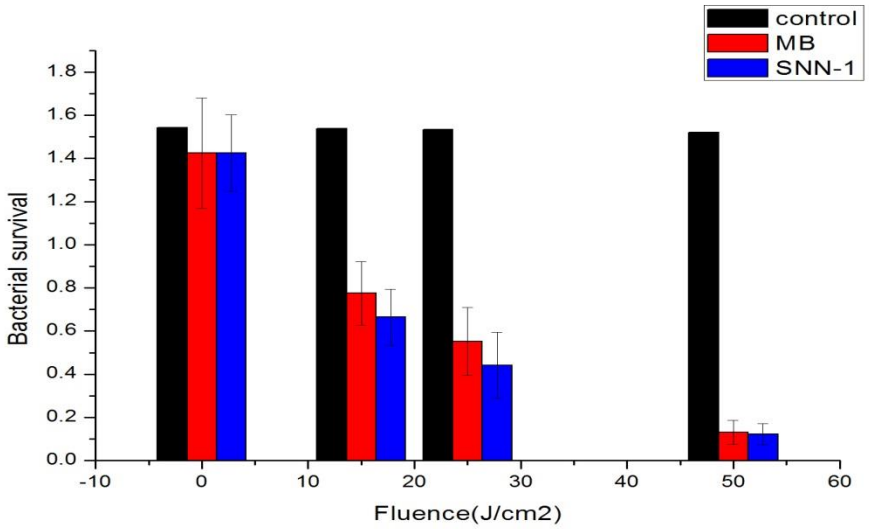

Fig.2a Histogram graphic representation of mean values and standard deviation of logarithmic of bacteria survival of E. coli planktonic suspensions according to the light fluence (0, 15, 25, 50J/cm^2).

The photodynamic antimicrobial effect of MB and SNN-1 against S. aureus (Bacterial viability) is presented in **Fig.2b**.The S. aureus bacterial growth was directly dependent on the light fluence (15, 25, 50J/cm^2). MB and SNN-1 promoted photokilling of S. aureus when irradiated with the light fluence of 50J/cm^2. When combined with the light fluence of 50J/cm^2, MB and SNN-1 caused 80% and 90% reduction of bacterial cell survival, respectively. The LC-control and 0J didn't cause a significant decrease of S. aureus bacterial growth and only the combination of PS and light caused the reduction of the survival.

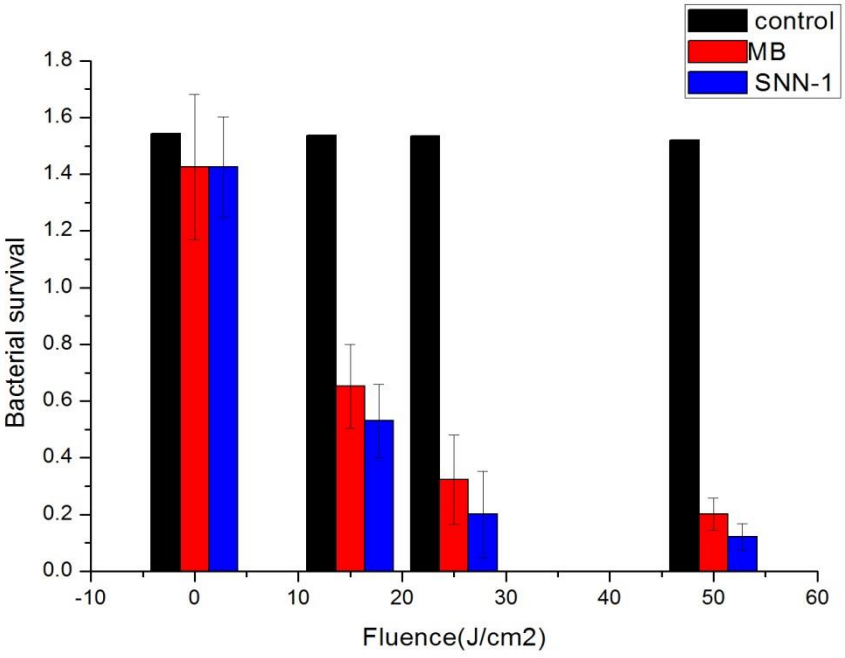

Fig.2b. Histogram graphic representation of mean values and standard deviation of logarithmic of bacteria survival of S. aureus planktonic suspensions according to the light fluence (0, 15, 25, 50J/cm^2).

The photodynamic antimicrobial effect of MB and SNN-1 against E. coli (Bacterial viability) is presented in **Fig.3a**. The E. coli bacterial growth was directly dependent on the concentration (15, 25, 50uM). MB and SNN-1promoted photokilling of E. coli when irradiated with the concentration of 15uM.When combined with the concentration of 15uM, MB and SNN-1 caused 50% and 60% reduction of E. coli bacterial cell survival, respectively. The NC-control and 0J didn't cause a significant decrease of E. coli bacterial growth and only the combination of PS and light reduced the number of colonies.

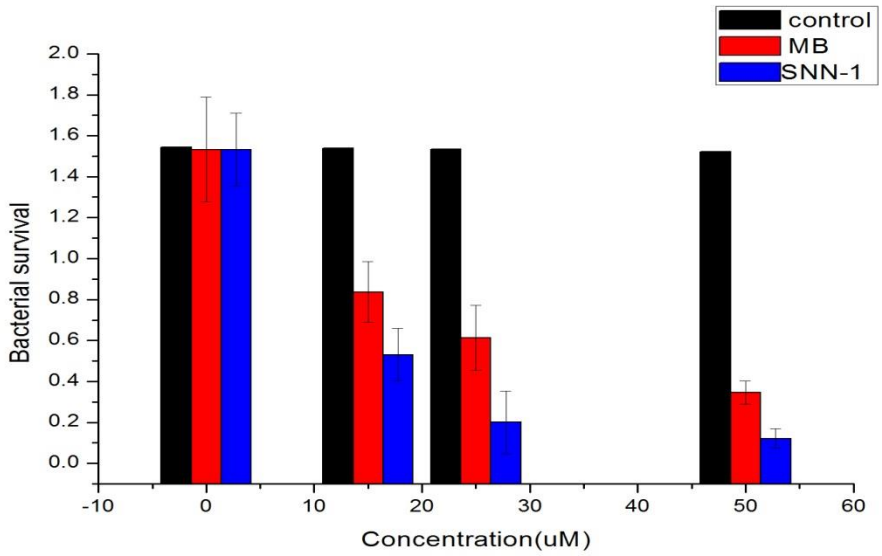

Fig.3a Histogram graphic representation of mean values and standard deviation of logarithmic of bacteria survival of E. coli planktonic suspensions according to the concentration of MB and SNN-1(15, 25, 50uM).

The photodynamic antimicrobial effect of MB and SNN-1 against S. aureus (Bacterial viability) is presented in **Fig.3b**. The S. aureus bacterial growth was directly dependent on the concentration (15, 25, 50uM). MB and SNN-1 promoted photokilling of S. aureus when irradiated with the concentration of 15uM. When combined with the concentration of 15uM, MB and SNN-1 caused 50% and 80% reduction of S. aureus bacterial cell survival, respectively. The NC and 0uM didn't cause a significant decrease of S. aureus bacterial growth and only the combination of PS and light caused the reduction of survival.

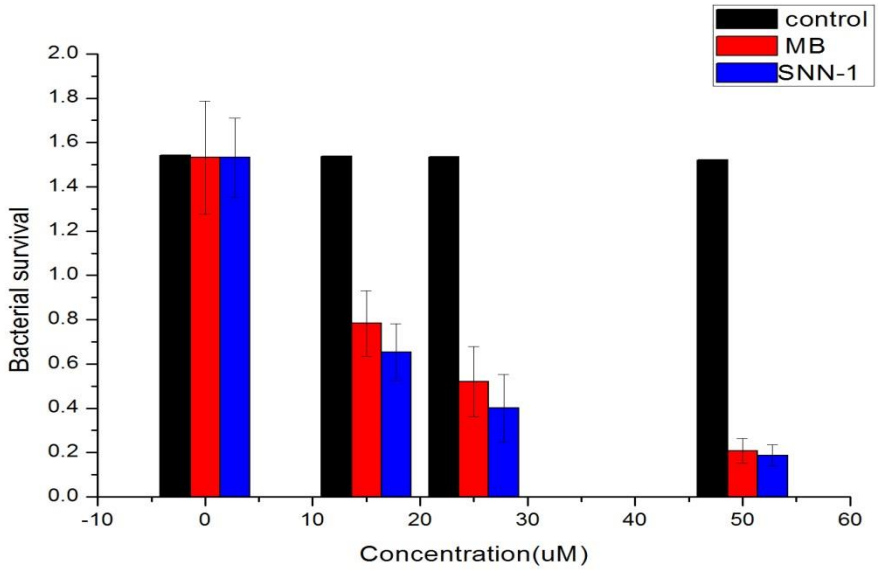

Fig.3b Histogram graphic representation of mean values and standard deviation of logarithmic of bacteria survival of S. aureus planktonic suspensions according to the concentration of MB and SNN-1(15, 25, 50uM).

DISCUSSIONS

In this paper, we show how photodynamic treatments affecting E. coli and S. aureus with MB and ADPM-06. The SNN-1 effects of photodynamic antimicrobial are well than the MB effects of photodynamic antimicrobial to E. coli. But the SNN-1 effects of photodynamic antimicrobial are worse than the MB effects of photodynamic antimicrobial to S. aureus in

vitro. Therefore, SNN-1is a promising photosensitizer for photodynamic antimicrobial therapy.

ACKNOWLEDGMENTS

The work was supported by Foundation of Shanghai Science and Technology Committee (No. 15431904100, 16ZR1400600, 17PJ1432800, 17431902600, 17430741800, 17430711900, 18430713000); The fundamental research fund for the central universities(No. 17D110513); The grant of Ningbo Science and Technology Bureau (No.2017BS1CA01008); China postdoctoral Science Foundation (63-1983).

REFERENCES

Bredell MG, Besic E, Maake C, Walt H (2010) The application and challenges of clinical PD-PDT in the head and neck region: a short review. J Photochem Photobiol B 101:185-190

Gallagher WM , Allen LT , O'Shea C. (2005) A potent non-porphyrin class of photodynamic therapeutic agent: cellular localization, cytotoxic potential and influence of hypoxia. J. British Journal of Cancer 92,1702-1710

Kathleen FernandesGrego (2017) Antimicrobial photodynamic therapy for infectious stomatitis in snakes: Clinical views and microbiological findings. J. Photodiagnosis Photodyn Ther 20: p. 196-200.

Konopka K, Goslinski T (2007) Photodynamic therapy in dentistry. J Dent Res 86:694-707

O'Connor AE, Mc Gee MM, Likar Y (2011) Mechanism of cell death mediated by a BF2-chelated tetraaryl-azadipyrromethene photodynamic therapeutic: dissection of the apoptotic pathway in vitro and in vivo. Int. J. Cancer: 130, 705-715

Wainwright M (2003) The use of dyes in modern biomedicine J. Biotechnic& Histochemistry 78:147-155

Wainwright M (1998) Photodynamic antimicrobial chemotherapy (PACT). J Antimicrob Chemother 42:13-28

Zolfaghari, Parjam S (2009) In vivo killing of Staphylococcus aureus using a light-activated antimicrobial agent. J. BMC Microbiology 1471-2180-9-27