Merocyanine 540-Mediated photodynamic therapy against *Staphylococcus aureus* Planktonic and Biofilm Cells

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Photodynamic inactivation of Staphylococcus aureus planktonic and biofilm cells by a photosensitizer, merocyanine 540 (MC 540) was investigated. For the planktonic experiments, the antimicrobial activity was enhanced with the increasing light dose, but an increase in light dose could not further improve the antimicrobial activity if the maximum excitation level attainable lay below the necessary minimum threshold level. A complete inactivation was achieved when the excitation level of MC 540 lay somewhere above the threshold level. The relationship between antimicrobial activity and the excitation level of MC 540 revealed that the more MC 540 was excited, the more S. aureus cells were killed. For the biofilm experiments, the antimicrobial activity was enhanced with the increasing light dose. No viable cells were detected when exposed to 15 µg/ml MC 540 and a light dose of 600 J/cm² or 20 µg/ml MC 540 and a light dose of 450 J/cm². The results from both planktonic and biofilm experiments suggest that the antimicrobial activity of photodynamic inactivation against S. aureus is closely related to the excitation level of MC 540.

INTRODUCTION

The formation and persistence of biofilms result in elevated transfer resistance, material deterioration and health risks [1]. In this study, we reported photodynamic inactivation (PDI) against *Staphylococcus aureus* planktonic and biofilm cells using an exogenous photosensitizer (PS), merocyanine 540 (MC 540). The aim of this study is to provide a quantitative description of MC 540-mediated PDI against *S. aureus* planktonic and biofilm cells and to demonstrate that the antimicrobial activity of PDI is closely related to the level of PS excitation.

RESULTS

MC 540-mediated PDI against *S. aureus* planktonic cells. Fig. 1 illustrates the survival ratio and the excitation level of MC 540 in response to different light doses. No antimicrobial activity was noted when the cells were irradiated without prior exposure to MC 540, regardless of the extent of irradiation. The survival ratio decreased with the increasing MC 540 concentrations and light dosages. All *S. aureus* cells became non-viable when exposed to MC 540 at a level of 15 µg/ml and when experiencing a light dose of 210 J/cm² or greater. Most of the MC 540 bound to cells was excited by only 50 J/cm² of irradiation. For the situation where bacterial cells were exposed to MC 540 at a level of 5 and 10 µg/ml, the excitation level of MC 540 increased with light dose and reached a plateau when the light dose was increased to a level greater than 50 J/cm². An increase in light dose could not further improve the antimicrobial activity of MC 540 if the maximum excitation level of MC 540 lay below 0.7 µg/10⁸ cells. By contrast, the antimicrobial activity of MC 540 revealed an enhancement with increasing light dose as long as the excitation level of MC 540 was greater than 0.82 µg/10⁸ cells, at which level, eventually, a complete kill of bacterial cells was achieved. Based upon the above observations, we hypothesized that a supra-threshold of excitation level of MC 540 is required to activate PDT and to result in complete antimicrobial activity.

MC 540-mediated PDI against *S. aureus* biofilms. Fig. 2 illustrates the survival fraction of MC 540-treated *S. aureus* biofilms in response to different light doses. No antimicrobial activity was found when the biofilms were irradiated without prior exposure to MC 540, regardless of the extent of irradiation. When treated with 5 µg/ml MC 540, the survival fraction of *S. aureus* biofilm cells decreased gradually with increasing irradiation which led to a 1.2-log reduction in the number of viable cells after exposure to 600 J/cm². A 2.1-log reduction in cell viability was found when cells were treated with MC 540 at a concentration of 10 µg/ml and exposed to a light dose of 450 J/cm². Similar to biofilms treated with 5 µg/ml, no further reduction in cell viability was achieved, even if the light dose was increased to 600 J/cm². When biofilms were treated with 15 µg/ml MC 540, a 2-log reduction was achieved with exposure to 300 J/cm². The survival fraction decreased dramatically as irradiation reached 450 J/cm² and no viable cells were detected when the light dose of 600 J/cm² was applied. All *S. aureus* cells were inactivated when exposed to 20 µg/ml MC 540 and a light dose of 450 J/cm² or greater.

Fig. 3 shows the excitation level of MC 540 bound to *S. aureus* biofilms in response to different light doses of irradiation. When treated with 5 µg/ml MC 540, the excitation level of MC 540 increased with increasing light doses but reached a plateau after exposure to 300 J/cm². When treated with 10 µg/ml MC 540, 16.7 IOD (integrated optical density, a unit of fluorescent intensity recorded by confocal laser scanning microscope)/µm²

excitation level was observed at a light dose of 600 J/cm². When treated with 15 µg/ml MC 540, the excitation level increased rapidly as irradiation increased up to 150 J/cm². When it exceeded 150 J/cm², the excitation level increased slowly with increasing light doses and reached 27.8 IOD/µm² after being treated with a 600 J/cm² light dose. When treated with 20 µg/ml MC 540, the same trend as treatment with 15 µg/ml MC 540 was found.

DISCUSSION

In biofilm cultures, complete inactivation of *S. aureus* biofilms was achieved by MC 540-mediated PDI. This might be attributed to a lack of relationship between PDI and biofilm resistance mechanisms, such as diffusion limitation, biocide neutralization and physiological adaptation. We hypothesized that a certain minimum threshold of excitation level of MC 540 was required for PDI to result in optimal antimicrobial activity. This study has revealed that, beyond a certain level of MC 540, an increase in light dose does not further improve the antimicrobial activity if the maximum excitation level attainable lays below the necessary minimum threshold level. To achieve the same PDI efficacy, a higher light dosage was required in biofilms than in planktonic cells. These phenomena should not be attributed to the incomplete penetration of MC 540, but might be due to the stratification of light exposure within biofilms. The results from both planktonic and biofilm experiments suggest that MC540-mediated PDI against *S. aureus* is a promising treatment.

REFERENCES

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Fig. 1. MC 540-mediated PDI against *S. aureus* planktonic cells: (a) antimicrobial activity; (b) MC540 excitation level in response to 0 (◇), 5 (●), 10 (□), 15 (▲), and 20 (▽) µg/ml MC 540.

Fig. 2. Antimicrobial activity of MC 540-mediated PDI against *S. aureus* biofilms. 0 (◇), 5 (●), 10 (□), 15 (▲), and 20 (▽) µg/ml MC 540.

Fig. 3. Excitation level of MC 540 bound to biofilms. Biofilms were treated with 5 (●), 10 (□), 15 (▲), and 20 (▽) µg/ml MC 540.