The Effect of Antimicrobial Photodynamic Therapy with Radachlorin® on Staphylococcus Aureus and Escherichia Coli: An in Vitro Study

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Abstract:

Introduction: The aim of this study is the evaluation of the effect of Antimicrobial Photodynamic Therapy with Radachlorin on Staphylococcus aureus and Escherichia coli. New windows are open in the antimicrobial field so-call Photodynamic therapy that incorporates a nonpoisonous photosensitizer (PS) with innocuous special wavelength photons to excite the PS.

Methods: Two strains of bacteria used in this study were Methicillin resistant Staphylococcus aureus (ATCC 33591; PTCC 1764) and Escherichia coli (ATCC 25922; PTCC1399). Concentrations of 0.2 ml of Radachlorin® were applied on 0.2 ml of bacterial suspensions and placed in a 48-well microtiter plate. The following groups were used: (I) L− PS− (no laser, no photosensitizer), (II) L−PS+ (treated only with PS), (III) L+ PS− (treated only with laser) and (IV) L+ PS+ (treated with laser and PS: photodynamic therapy group). Aliquots of bacterial suspensions were sensitized with Radachlorin® for 15 minutes in the dark at room temperature and then bacterial suspensions in group III and IV were irradiated with 210 mW (power density) and 12 J/cm2 (energy density) on continuous mode.

Results: This study showed that photodynamic therapy reduces 0.14 log 10 in E.Coli (group IV) and there were significant differences for group IV (P<0.01). Photodynamic therapy in S.Aureus showed 6.28 log 10 colony count reduction (group IV) and there were highly significant differences in Photodynamic therapy group (P<0.0001).

Conclusion: Radachlorin® have bactericidal effect on S.aureus (6.28 log 10) and bacteriostatic effect on E.coli (0.14 log 10).

Keywords: photodynamic therapy; staphylococcus aureus; escherichia coli

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Introduction

Presently, two of the overt multidrug-resistant pathogens causing worldwide worry are Methicillin-resistant Staphylococcus aureus (MRSA) and “extended-spectrum β-lactamase (ESBL) “producing Escherichia coli 1,2. Some studies showed significant increasing trends of S.Aureus and E.Coli in urinary tract infections (UTI), respiratory tract infections (RTI), and surgical site infections (SSI) 3,4. Many efforts have been done to overcome the pathogens such as producing new antibiotics but the microorganisms are wily and by different methods
annihilate the antibiotics. New windows are open in antimicrobial field so-call Photodynamic therapy that incorporates a nonpoisonous photosensitizer (PS) with innocuous special wavelength photons to excite the PS to its reactive triplet state, which will then produce reactive oxygen species, such as superoxide and singlet oxygen that are poisonous to cells and kill them. In many research, chlorin e6 as a photosensitiser has been used generally. Radachlorin® which is a chlorophyll derivative, including sodium chloride e6, chlorine p6, purpurine 5, that have been successfully used in tumors diagnosis and tumors treatment. There have been only few studies on the antimicrobial effects of Radachlorin®, although there have been several studies on chlorin e6, which is a major component of Radachlorin®. Fekrazad et al reported that the combination of Radachlorin® and laser was more effective on Streptococcus mutans than Radachlorin® or laser alone (p<0.05). Vahabi et al reported an in vitro study that toluidine blue O (TBO) mediated photodynamic therapy seems to be more efficient than Radachlorin® in reducing the viability of Streptococcus mutans. We can’t find any reaseach on Staphylococcus aureus and Escherichia coli with Radachlorin®. The main purpose of this study was to explore the antimicrobial photodynamic therapy effect of Radachlorin® on Persian Type Culture Collection (PTCC) S. aureus and E. coli.

**Methods**

**Bacteria**

Two strains of bacteria used in this study were Methicillin resistant Staphylococcus aureus; Persian Type Culture Collection (PTCC 1764) and Escherichia coli (PTCC 1399). These bacteria were maintained by weekly subculture on nutrient agar (Merck). These bacteria were grown in brain-heart infusion broth in an orbital shaker at 37°C for 24 h. An aliquot of this suspension was then added to nutrient broth and grown to mid-log phase (OD600=0.6, 10⁸ cells/mL).

**Photosensitizers and laser sources**

Radachlorin® gel (0.1%, 25 g) was obtained from RADA-FARMA Ltd, Russia and stored at 0–8 °C in the dark. The laser source used was a diode laser (Milon-LAHTA, Russia) with a fiber optic diameter of 800 micrometer, a maximum output of 2.5 W and a predominant wavelength of 662 nm.

**Photodynamic therapy**

Preparation of suspension of microbial cells was performed, preparation of liquid media (brain-heart infusion broth, BHI, for bacteria) and autoclave. Preparation of solid media was performed by addition of 1.5% microbiological agar to above broth and poured into 10 × 10 cm square petri dishes. Concentration of 0.2 ml of Radachlorin® was applied on 0.2 ml of the bacterial suspensions and placed in a 48-well microtiter plate. The following groups were used: (I) L− PS− (no laser, no photosensitizer), (II) L−PS+ (treated only with PS), (III) L+ PS− (treated only with laser) and (IV) L+ PS+ (treated with laser and PS: photodynamic therapy group). Aliquots of bacterial suspensions were sensitized with Radachlorin® for 15 minutes in the dark at room temperature and then bacterial suspensions in group III and IV were irradiated with continuous mode, 23 second, 213 mW (power density) and 12 J/cm² (energy density). The focal point of laser was matched by one of 48-well microtiter plate and the fiber optic of the laser was at 1mm above the microtiter plate. The plates were incubated at 37°C overnight. The laboratory technician was blinded to the study and the numbers of colonies was counted to determine the survival fractions.

**Statistical analysis**

Values were expressed as log 10 means±standard deviation. Comparisons between means of groups were used as well as the univariate analysis of variance and Post P<0.05 was considered statistically significant.

**Results**

This study showed that photodynamic therapy reduces 0.14 log 10 in E.Coli (group IV) and there were significant differences for group IV (P<0.01) (Table 1) but no differences in other groups and conrol group (group I) were obtained.

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean</th>
<th>Std. Deviation</th>
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<tr>
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<td>.06542</td>
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<tr>
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<td>(IV)</td>
<td>7.0105</td>
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Table 1. Mean value log 10 E.coli colony count for Radachlorin®, (I) L− PS− (no laser, no photosensitizer), (II) L−PS+ (treated only with PS), (III) L+ PS− (treated only with laser) and (IV) L+ PS+ (treated with laser and PS: photodynamic therapy group)
mean values in each group (Figure 1).

Photodynamic therapy in S.Aureus showed 6.28 log 10 colony count reduction (group IV) and there were highly significant differences in Photodynamic therapy group (P<0.0001) and other groups (Table 2).

Other groups of S.Aureus (group II and III) showed no significant differences in comparison to the control group. Profile pilot diagram of S.Aureus shows colony count mean values in each group (Figure 2).

For analysis of difference between E.Coli and S.Aureus Bactericidal activity of an antimicrobial agents means >3 log10 reduction of bacterial counts and Bacteristatic activity of one antimicrobial agents means <3 log10 reduction of bacterial counts [16]. According to this study Radachlorin® has Bactericidal effect on S.aureus (6.28 log 10) and Bacteristatic effect on E.coli (0.14 log 10).

Discussion

The ability of Radachlorin® to act as a photosensitizer after irradiation with laser photons has been demonstrated in a few studies but several studies were done on chlorin e6, which is a major component of Radachlorin® [8-12]. In the present study, we examined the antimicrobial effect of Radachlorin® mediated PDT against S.aureus and E.coli. Statistical analysis showed that Radachlorin® mediated PDT is very effective in inhibiting the growth of S. aureus. Bactericidal activity of an antimicrobial agents means >3 log10 reduction of bacterial counts and Bacteristatic activity of one antimicrobial agents means <3 log10 reduction of bacterial counts [16]. According to this study Radachlorin® has Bactericidal effect on S.aureus (6.28 log 10) and Bacteristatic effect on E.coli (0.14 log 10). Several studies showed that Gram negative bacteria are largely resistant to antimicrobial photodynamic therapy due to their special cell wall structure [17,18].

Figure 1. Mean value log 10 E.coli colony count, (I) L− PS− (no laser, no photosensitizer), (II) L−PS+ (treated only with PS), (III) L+ PS−(treated only with laser) and (IV) L+ PS+ (treated with laser and PS: photodynamic therapy group)

Figure 2. Mean value log 10 S.Aureus colony count, (I) L− PS− (no laser, no photosensitizer), (II) L−PS+ (treated only with PS), (III) L+ PS−(treated only with laser) and (IV) L+ PS+ (treated with laser and PS: photodynamic therapy group)

Table 2. Mean value log 10 S.Aureus colony count for Radachlorin®, (I) L− PS− (no laser, no photosensitizer), (II) L−PS+ (treated only with PS), (III) L+ PS−(treated only with laser) and (IV) L+ PS+ (treated with laser and PS: photodynamic therapy group)
Conclusion

Radachlorin® have Bactericidal effect on S.aureus (6.28 log 10) and Bacteristatic effect on E.coli (0.14 log 10).

Acknowledgments

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Disclosure Statement

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References