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In vitro bactericidal activity of blue light (465 nm) phototherapy on meticillin-susceptible and meticillin-resistant *Staphylococcus pseudintermedius*

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Background – *Staphylococcus pseudintermedius* is the most common cause of bacterial skin infections in dogs. Meticillin-resistant infections have become more common and are challenging to treat. Blue light phototherapy may be an option for treating these infections.

Hypothesis/Objectives – The objective of this study was to measure the *in vitro* bactericidal activity of 465 nm blue light on meticillin-susceptible *Staphylococcus pseudintermedius* (MSSP) and meticillin-resistant *Staphylococcus pseudintermedius* (MRSP). We hypothesized that irradiation with blue light would kill MSSP and MRSP in a dose-dependent fashion *in vitro* as previously reported for meticillin-resistant *Staphylococcus aureus* (MRSA).

Methods – In six replicate experiments, each strain [MSSP, $n = 1$; MRSP ST-71 (KM1381) $n = 1$; and MRSA (BAA-1680) $n = 1$] were cultivated on semisolid media, irradiated using a 465 nm blue light phototherapeutic device at the cumulative doses of 56.25, 112.5 and 225 J/cm^2 and incubated overnight at 35°C. Controls were not irradiated. Colony counts (CC) were performed manually. Descriptive statistics were performed and treatment effects assessed using the Wilcoxon–Mann–Whitney rank-sum test. Bonferroni-corrected rank-sum tests were performed for *post hoc* analysis when significant differences were identified.

Results – There was a significant decrease in CC with blue light irradiation at all doses for MRSA ($P = 0.0006$) but not for MSSP ($P = 0.131$) or MRSP ($P = 0.589$).

Conclusions – Blue light phototherapy significantly reduced CC of MRSA, but not of MSSP or MRSP. The mechanism for the relative photosensitivity of the MRSA isolate is unknown, but is hypothesized to be due to an increased concentration of porphyrin in *S. aureus* relative to *S. pseudintermedius*, which would modulate blue light absorption.

Introduction

Staphylococcus pseudintermedius is a common commensal and opportunistic pathogen of the skin of dogs and is the most common cause of bacterial skin infections.[1] In recent years, meticillin-resistant *S. pseudintermedius* (MRSP) infections have become much more common.[2] The prevalence in the USA of MRSP in clinical samples from dogs with canine pyoderma ranges from 15.6 to 38.2%.[3,4] With the emergence of MRSP the number of oral antimicrobial drugs to which bacterial isolates are susceptible is limited, often leading to pharmacotherapeutic choices that have profound adverse effects or are reserved for human infections.[5] Topical antimicrobial therapy has been shown to be effective for treatment of some MRSP infections,[6] but these treatments can be time consuming and inconvenient for owners. There is a need to develop new therapies to treat these infections that are both effective and have minimal adverse effect potential.

Photostimulation is the use of light to activate biological cells or tissues. Therapy with photostimulation is called phototherapy, light therapy or photomodulation. Photostimulation can be performed using light emitting diodes (LEDs) which produce a narrow spectrum of light in an incoherent manner, where the light is randomly spread out once emitted from the light source.[7] LEDs have different depth of penetration based on their wavelength and can affect cellular metabolism by triggering intracellular photobiochemical reactions.[7] Wavelengths available in commercial LED units include ultraviolet (100–400-nm),[8,9] blue (400–470-nm)[7], yellow (570–590-nm)[7], red (630–700-nm)[7] and infrared (800–1200-nm).[7] The deepest target of LED light penetration varies. In humans blue light

Abbreviations: 5-ALA, 5-aminolevulinic acid; CC, colony counts; LEDs, light emitting diodes; MALDI-TOF, Matrix Assisted Laser Desorption Ionization Time-of-Flight; MRSA, meticillin-resistant *Staphylococcus aureus*; MRSP, meticillin-resistant *Staphylococcus pseudintermedius*; MSSA, meticillin-susceptible strain; MSSP, meticillin-susceptible *Staphylococcus pseudintermedius*; PBS, phosphate buffered saline; PDT, Photodynamic therapy; TSA, tryptic soy agar.

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targets the epidermis (less than 1 mm), yellow light the papillary dermis (0.5–2 mm), red light the adnexa (2–3 mm) and infrared light (5–10 mm) both the adnexa and reticular dermis.[7] These depth penetrations would be expected to be similar in animal skin.

Blue light phototherapy has been shown to be a treatment option for bacterial infections. Although the exact mechanism is unknown, blue light is thought to excite intracellular porphyrins and produce cytotoxic reactive oxygen species (ROS).[10] These ROS have an antimicrobial effect on the bacteria, but are not detrimental to the host cells.[10] Blue light phototherapy has been shown to be bactericidal against *Staphylococcus* species *in vitro*.[11–14] A dose-dependent bactericidal effect was observed on *Staphylococcus aureus*, with the greatest reduction of bacteria (62%) at the highest dose of 15 J/cm^2 using 470 nm blue light.[11] Optimal doses of 470 nm blue light phototherapy have been reported to be between 55 and 220 J/cm^2, resulting in 90.4–100% reduction of MRSA.[11–14] The bacterial densities of MRSA in these studies ranged from 3×10^6 colony forming units (cfu)/mL up to 7×10^6 cfu/mL.[12–14] Higher densities (7×10^6 cfu/mL), reflecting increasing bacterial loads, required higher doses.[12]

Because blue light phototherapy has been shown to be effective in killing bacteria associated with human staphylococcal infections, it may be a useful adjunctive or sole therapy option for staphylococcal infections in dogs, specifically meticillin-resistant infections. As there are no published studies evaluating the bactericidal effect of blue light phototherapy on MRSP or meticillin-susceptible *S. pseudintermedius* (MSSP), the objective of this study was to determine the *in vitro* bactericidal activity of 465 nm blue light on MSSP and MRSP. We hypothesized that irradiation with 465 nm blue light would suppress growth of MSSP and MRSP in a dose-dependent manner, as reported previously for MRSA.[13,14]

Materials and methods

Bacterial isolates and culture

A sequence-typed strain of MRSP ST-71 (KM1381), a sequence-typed strain of USA300 MRSA (BAA-1680) and an untyped clinical MSSP isolate were selected for use as the test isolates. The MRSP ST-71 (KM1381) isolate was obtained from the University of Tennessee College of Veterinary Medicine bacteriology laboratory in Knoxville, TN, USA.[15,16] The USA300 reference strain of MRSA was obtained from American Type Culture Collection (ATCC® BAA-1680).[17] The clinical MSSP isolate was speciated using Matrix Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF) mass spectrometry.[18] A standardized inoculum of 15 μL of approximately 10^4 cfu/mL of each isolate was spread plated onto 35 mm petri dishes containing tryptic soy agar (TSA). This plate size was chosen to attain uniform irradiation as the blue light was emitted from an opening that was approximately 25 mm in diameter. Briefly, a 0.5 McFarland standard suspension (approximately 10^8 cfu/mL concentration) was made for each isolate in sterile water. Ten-fold serial dilutions were performed by placing 40 μL of the bacterial suspension in 360 μL of sterile phosphate buffered saline (PBS) to reach a final dilution of approximately 10^4 cfu/mL. Dilutions were obtained to achieve a range of 10–100 cfu on the 35 mm TSA plates. Plates for the treatment groups (MSSP, MRSP and MRSA isolates) were inoculated with 15 μL of the 10^4 cfu/mL dilution and spread onto the plates. Control plates for each isolate were spread in the same manner. After inoculation, the treatment group plates were irradiated with blue light (see below) and incubated overnight at 35°C in ambient air. Plates for the controls were not irradiated prior to incubation. Incubation conditions (time and temperature) were identical for control and treated plates.

Blue light irradiation

A 465 nm blue light therapeutic device (MR4 ACTIVet PRO™ device, Multi Radiance Medical®; Solon, OH, USA) was used for all irradiations (Table 1). The device was clamped approximately 6 mm above the TSA plates for even dispersion of light across the inoculated area (Figure 1). Treatment groups (MSSP, MRSP and MRSA) were irradiated once with the following doses: 56.25 J/cm^2 (15 min exposure), 112.5 J/cm^2 (30 min exposure) and 225 J/cm^2 (60 min exposure). The controls were not irradiated. The three doses were chosen based on a previous study in which irradiation of USA300 MRSA (ATCC® BAA-1680) with 470 nm blue light at 55, 110 and 220 J/cm^2 produced 69–92%, 80–100% and 100% suppression of bacterial growth, respectively.[13] Each dose was repeated in sextuplicate for each isolate. Colony counts (the total numbers of colonies on each individual plate; CC) were performed manually.

Statistical analysis

Descriptive statistics were generated for CC for control and treatment groups at each dose and the data were tested for distribution and normality by visual inspection and with the D'Agostino – Pearson test (MedCalc® for Windows, v15.0; Ostend, Belgium). As data were not normally distributed, the results are displayed as medians, interquartile ranges and ranges (MedCalc® for Windows, v15.0) and percentage reduction for CC.

Data for each of the three doses was also combined for each bacterial isolate and the control and treatment groups were then compared using a Wilcoxon–Mann–Whitney rank-sum test (SPSS IBM ve24; Armonk, NY, USA). When a significant difference was identified, Bonferroni-corrected rank-sum tests (SPSS IBM v24) were performed at each dose for *post hoc* analysis. Statistical significance was set at $P < 0.05$.

Results

The median CC and percentage reduction of the treatment groups (MSSP, MRSP, MRSA) after irradiation with 465 nm blue light at 56.25, 112.5 and 225 J/cm^2 are shown in Table 2. The percentage reduction of CC was greatest for MRSA at all doses, reaching 100% at the two highest doses. In contrast, the percentage reduction of CC for MSSP and MRSP was minimal with the maximum reduction found at the highest dose (225 J/cm^2) of 11.7% for MSSP and 21.2% for MRSP.

Table 1. Properties of the blue light phototherapeutic device (MR4 ACTIVet PRO™, Multi Radiance Medical®; Solon, OH, USA)

Number of blue LEDs	3
Wavelength of blue LEDs (nm)	465 (±10)
Mode	Continuous
Average optical output (mW) – each LED	83.33
Power density (mW/cm^2) – each LED	111.11
Energy density (J/cm^2) – each LED	100, 200, 400
Dose (J) – each LED	75, 150, 300
Spot size of blue LED (cm^2)	0.75
Magnetic Field (mT)	45
Irradiation time (s)	**900, 1800, 3600**
Total dose (J)	225, 450, 900
Aperture of device (cm^2)	4
Energy density at aperture (J/cm^2)	**56.25, 112.50, 225.00**
Power density at aperture (mW/cm^2)	62.50

Energy density at aperture and irradiation times used in the study are in bold.

Figure 1. The 465 nm blue light therapeutic device (MR4 ACTIVet PRO™ device, Multi Radiance Medical®; Solon, OH, USA) irradiating a 35 mm petri dish containing tryptic soy agar plated with a bacterial isolate.

The effects of dose on CC for the treatment groups is shown in Figure 2; there was a significant difference in CC for MRSA between treatment and control groups at each dose ($P = 0.006$).

A significant reduction in combined median MRSA CC after treatment ($P < 0.0005$) for all doses was present. There was no significant reduction in median CC after irradiation for MSSP ($P = 0.131$) or in median CC for MRSP ($P = 0.589$) (see Table S1 in Supporting Information).

Representative plates are depicted in Supporting Information Figure S1.

Discussion

Blue light 465 nm phototherapy significantly reduced CC for MRSA, but not for MSSP or MRSP. An earlier study using 470 nm blue light phototherapy reported a significant dose-dependent reduction in MRSA colony counts with 90.4% of the colonies killed at a dose of 55 J/cm^2.[14] However, 100% reduction was not obtained, even at the highest dose tested (60 J/cm^2).[14] Our study had similar results for MRSA with a 93.3% CC reduction at 56.25 J/cm^2. In two other studies, 470 nm blue light suppressed MRSA at 55 J/cm^2; however, to achieve 100% bacterial suppression irradiation had to be performed twice for standard or less dense cultures. Denser cultures, when irradiated once, required the highest dose of 220 J/cm^2 to achieve 100% kill.[12,13] Likewise, in our study, the two highest doses, 112.5 and 225 J/cm^2 were required to achieve 100% reduction in CC for MRSA.

Although *S. pseudintermedius* is the most common organism isolated in dogs with bacterial skin infections,[1] MRSA infections are an emerging problem in veterinary medicine.[2,19,20] As hand-held LED blue light phototherapic devices are commercially available, blue light phototherapy may be an effective option for treating MRSA. Based on the results of our study, to achieve 100% kill, treatment durations would need to be at least 30 min, so this therapy would be best suited for localized rather than generalized infections. The effect of factors such as hair and organic debris need to be considered for use *in vivo*. Clipping the hair and cleaning the skin over the area to be irradiated with alcohol has been shown to increase the depth of penetration of the light when a low-level laser therapy probe was used on the flexor tendon in the horse.[21] In an *in vivo* mouse model of MRSA skin abrasion infections, 415 nm blue light delivered at 108 J/cm^2 rapidly reduced the bacterial burden, suggesting the use of blue light may be an option for treatment of MRSA skin infections.[22]

Interestingly, the percentage CC reductions for MSSP and MRSP were minimal, with the greatest reduction of 11.7 and 21.2% respectively at the highest dose of 225 J/cm^2. These results were not expected because blue light phototherapy had been effective *in vitro* against MRSA in previous studies[12–14] and in ours. Although the mechanism of action of blue light is not fully understood, it is believed to excite intracellular porphyrins, thus generating the production of cytotoxic ROS that kill the bacteria.[10] Because porphyrins are a key factor in absorption of blue light, it is possible that differences in the amount of endogenous porphyrins between bacterial strains would result in differences in blue light absorption and killing of the bacteria. One study compared the difference in absorption of visible light (400–800 nm) of two different strains of *S. aureus*, one meticillin-susceptible strain (MSSA) and one resistant strain (MRSA).[23] The MSSA strain had a maximum reduction in bacterial viability of 99.8% compared to 55.5% for MRSA. Porphyrins were extracted and the relative fluorescence measured using a spectrometer. The MSSA had a higher porphyrin concentration and cytotoxic oxygen radical production compared to MRSA which likely accounted for greater absorption of light and a greater reduction of the bacteria.[23] The relative percentage production of porphyrin also has been measured in *S. aureus* using high performance liquid

Table 2. Median colony counts and percentage reduction for control group and treatment groups [meticillin-susceptible *Staphylococcus pseudintermedius*, meticillin-resistant *S. pseudintermedius* (KM1381) and meticillin-resistant *S. aureus* (BAA-1680)] after irradiation with 465 nm blue light

	MSSP			MRSP (KM1381)			MRSA (BAA-1680)		
	CC			CC			CC		
Blue light dose	CG	TG	% Red	CG	TG	% Red	CG	TG	% Red
56.25 J/cm^2	29	27	6.9	23	25	−8.7	67	4.5	93.3
112.5 J/cm^2	31	28	9.7	29.5	29.5	0	72.5	0	100
225 J/cm^2	38.5	34	11.7	26	20.5	21.2	90.5	0	100

MSSP meticillin-susceptible *Staphylococcus pseudintermedius,* MRSP meticillin-resistant *S. pseudintermedius,* MRSA meticillin-resistant *S. aureus,* CC (median) colony counts, CG control group (not irradiated), TG treatment group, % Red percentage reduction.

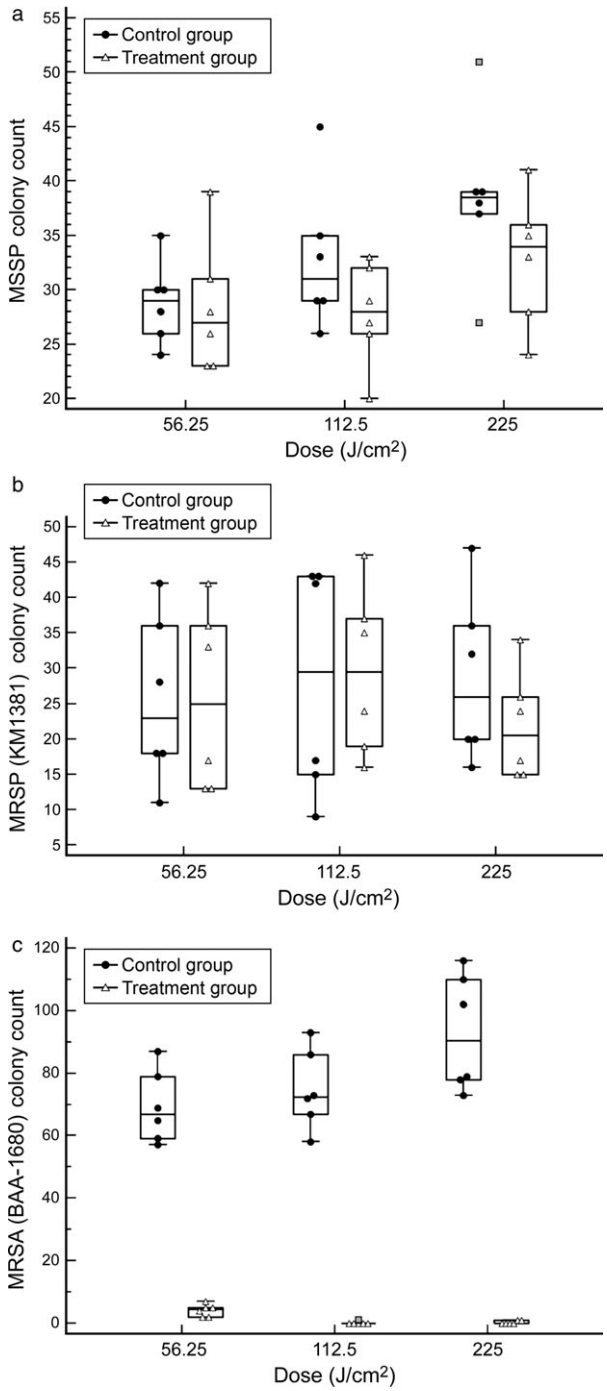

Figure 2. Box plots showing the bacterial colony counts at each dose (56.25, 112.5, 225 J/cm^2) for the control (not irradiated) and treatment groups (irradiated with 465 nm blue light) for (a) MSSP, (b) MRSP (KM1381) and (c) MRSA (BAA-1680). Black circles and open triangles represent the colony counts of each individual plate (grey squares are outliers). There was a significant difference in CC for MRSA between treatment and control groups at each dose ($P = 0.006$). MSSP meticillin-susceptible *Staphylococcus pseudintermedius,* MRSP meticillin-resistant *S. pseudintermedius,* MRSA meticillin-resistant *S. aureus*.

chromatography.[24] To the best of the authors' knowledge, there are no studies of endogenous porphyrin concentration measurement in MSSP or MRSP.

Photodynamic therapy (PDT) is a treatment option that involves photosensitization of a target using a topical or systemic agent that is activated by light in the presence of oxygen and produces a cytotoxic reaction.[25] PDT can be performed using 5-aminolevulinic acid (5-ALA), a natural amino acid that is the precursor of a strong photosensitizer, protoporphyrin IX within cells.[25] 5-ALA can be administered locally, systemically (intravenous and intraperitoneal) and orally. 5-ALA-PDT combined with a 410 nm LED had an antibacterial effect on MRSA *in vitro* with a 5 $\log_{10}$-unit decrease in organisms at 50 J/cm^2. In the same study, in a mouse model of MRSA-infected wounds, the use of 5-ALA-PDT with a 410 nm LED accelerated wound healing and decreased bacterial counts on the wound surface.[25] 5-ALA-PDT may be an option both *in vitro* and *in vivo* for MRSP and MSSP to help increase bacterial kill.

In conclusion, blue light phototherapy significantly reduced CC of MRSA, but not of MSSP or MRSP. The mechanism for the relative photosensitivity of the MRSA isolate is unknown, but is hypothesized to be due to an increased concentration of porphyrin in *S. aureus* relative to *S. pseudintermedius*, which would modulate blue light absorption. Further studies are needed to measure the concentration of porphyrins and assess porphyrin relevance in blue light absorption in MSSP and MRSP, as well as to assess the use of PDT combined with blue light for MSSP and MRSP infections.

Acknowledegments

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Figure S1. Representative culture plates of meticillin-susceptible *Staphylococcus pseudintermedius* (MSSP) (a–d), meticillin-resistant *S. pseudintermedius* (MRSP) (KM1381) (e–h) and meticillin-resistant *S. aureus* (MRSA) (BAA-1680) (i–l) irradiated with 56.25, 112.5 and 225 J/cm^2 465 nm blue light. Control plates (a,e,i) were not irradiated.

Table S1. Combined median, interquartile range and range of colony counts for control groups and treatment groups [MSSP meticillin-susceptible *Staphylococcus pseudintermedius*, MRSP meticillin-resistant *S. pseudintermedius* (KM1381) and MRSA meticillin-resistant *Staphylococcus aureus* (BAA-1680)] after irradiation with 465 nm blue light.

Résumé

Contexte – *Staphylococcus pseudintermedius* est la cause la plus fréquente d'infection bactérienne cutanée chez le chien. Les infections méticilline-résistantes sont devenues fréquentes et sont un défi thérapeutique. La photothérapie en lumière bleue pourrait être une option dans le traitement de ces infections.

Hypothèses/Objectifs – L'objectif de cette étude était de mesurer l'activité bactéricide *in vitro* de la lumière bleue de 465 nm sur les MSSP (*Staphylococcus pseudintermedius* sensible à la méticilline) et MRSP (*Staphylococcus pseudintermedius* résistant à la méticilline). Nous supposons que l'irradiation en lumière bleue pourrait tuer les MSSP et MRSP de manière dose-dépendante *in vitro* comme précédemment décrit pour les MRSA (*Staphylococcus aureus* résistant à la méticilline).

Méthodes – Dans six expérimentations répétées, chaque souche [MSSP, $n = 1$; MRSP ST-71 (KM1381) $n = 1$; et MRSA (BAA-1680) $n = 1$] était cultivée sur milieu semi-solide, irradiée par un appareil photo-thérapeutique en lumière bleue de 465 nm à des doses cumulés de 56.25, 112.5 et 225 J/cm^2 et incubées à 35°C pendant la nuit. Les contrôles n'étaient pas irradiés. Le comptage des colonies (CC) ont été réalisés manuellement. Les statistiques descriptives ont été réalisées et les effets du traitement évalués par le test Wilcoxon–Mann–Whitney rank-sum. Les tests de Bonferroni-corrected rank-sum ont été réalisés pour les analyses post hoc lorsque des différences significatives étaient identifiées.

Résultats – Il y avait une diminution significative des CC en irradiation en lumière bleue pour toutes les doses pour MRSA ($P = 0.0006$) mais pas pour MSSP ($P = 0.131$) ou MRSP ($P = 0.589$).

Conclusions – La photothérapie de lumière bleue a significativement diminué le CC de MRSA mais pas de MSSP ou MRSP. Le mécanisme pour la photosensibilité relative des souches de MRSA n'est pas connu mais il est supposé qu'il pourrait être dû à une concentration accrue de porphyrine dans les *S. aureus* comparé *S. pseudintermedius*, qui régulerait l'absorption de la lumière bleue.

Resumen

Introducción – *Staphylococcus pseudintermedius* es la causa más común de infecciones bacterianas de la piel en perros. Las infecciones resistentes a la meticilina se han vuelto más comunes y son difíciles de tratar. La fototerapia con luz azul puede ser una opción para tratar estas infecciones.

Hipotesisi/Objetivos – El objetivo de este estudio fue medir la actividad bactericida *in vitro* de 465 nm de luz azul sobre *Staphylococcus pseudintermedius* (MSSP) sensible a la meticilina y *Staphylococcus pseudintermedius* resistente a meticilina (MRSP). La hipótesis era que la irradiación con luz azul mataría a MSSP y MRSP de forma dependiente de dosis *in vitro* como se ha descrito anteriormente para *Staphylococcus aureus* resistente a meticilina (MRSA).

Métodos – En seis experimentos repetidos, cada cepa [MSSP, *n* = 1; MRSP ST-71 (KM1381) *n* = 1; y MRSA (BAA-1680) *n* = 1] se cultivaron en medio semisólido, se irradiaron utilizando un dispositivo fototerapéutico de luz azul de 465 nm a dosis acumuladas de 56,25, 112,5 y 225 J/cm2 y se incubaron durante toda la noche a 35°C. Los controles no fueron irradiados. Los recuentos de colonias (CC) se realizaron manualmente. Se realizaron las descripciones estadísticas y se evaluaron los efectos del tratamiento utilizando la prueba de suma de rangos de Wilcoxon-Mann-Whitney. También se realizó una prueba corregida de suma de rangos de Bonferroni para análisis post hoc cuando se identificaron diferencias significativas.

Resultados – Hubo una disminución significativa de CC con irradiación de luz azul en todas las dosis para MRSA (*P* = 0,0006) pero no para MSSP (*P* = 0,131) o MRSP (*P* = 0,589).

Conclusiones – La fototerapia con luz azul redujo significativamente el CC de MRSA, pero no de MSSP o MRSP. El mecanismo para la fotosensibilidad relativa del aislado de MRSA es desconocido, pero se supone que se debe a un aumento de la concentración de porfirina en *S. aureus* en relación con *S. pseudintermedius*, que modularía la absorción de la luz azul.

Zusammenfassung

Hintergrund – *Staphylococcus pseudintermedius* ist die häufigste Ursache für bakterielle Hautinfektionen bei Hunden. Methicillin-resistente Infektionen werden immer häufiger und sind eine Herausforderung bei der Behandlung. Blaulichtphototherapie könnte eine Behandlungsoption für diese Infektionen darstellen.

Hypothese/Ziele – Das Ziel dieser Studie war eine Messung der *in vitro* bakteriziden Aktivität des 465 nm Blaulichts auf Methicillin-empfindliche *Staphylococcus pseudintermedius* (MSSP) und Methicillin-resistente *Staphylococcus pseudintermedius* (MRSP). Wir hypothetisierten, dass eine Bestrahlung mit Blaulicht MSSP und MRSP in einer Dosis-abhängigen Weise *in vitro* abtöten würde, wie es schon früher für Methicillin-resistente *Staphylococcus aureus* (MRSA) beschrieben worden war.

Methoden – In sechs replizierten Experimenten wurde jeder Stamm [MSSP, *n*=1; MRSP ST-71 (KM1381) *n* = 1; und MRSA (BAA-1680) *n* = 1] auf halbfesten Medien kultiviert, und mit einer 465 nm starken photodynamischen Blaulichteinrichtung mit kumulativen Dosen von 56,25; 112,5 und 225 J/cm^2 bestrahlt und über Nacht bei 35°C inkubiert. Die Kontrollen wurden nicht bestrahlt. Die Kolonien (CC) wurden manuell ausgezählt. Eine deskriptive Statistik wurde durchgeführt und Behandlungseffekte mittels Wilcoxon-Mann-Whitney Rangsummentest beurteilt. Wenn signifikante Unterschiede identifiziert wurden, wurde der korrigierte Bonferroni Rangsummentest zur post hoc Analyse durchgeführt.

Ergebnisse – Es gab eine signifikante Verminderung der CC bei allen Dosierungen der Blaulichtbestrahlung bei MRSA (*P* = 0,0006), aber nicht bei MSSP (*P* = 0,131) oder MRSP (*P* = 0,589).

Schlussfolgerungen – Blaulichtphototherapie reduzierte die CC der MRSA, aber nicht jene von MSSP oder MRSP, signifikant. Der Mechanismus der relativen Photosensibilität der MRSA Isolate ist unbekannt, aber es wird hypothetisiert, dass die Ursache in einer erhöhten Konzentration des Porphyrins im *S. aureus* im Vergleich zum *S. pseudintermedius* liegt, welche die Blaulichtabsorption modulieren könnte.

要約

背景 – *Staphylococcus pseudintermedius*は、犬の細菌性皮膚感染症の最も一般的な原因菌である。メチシリン耐性感染症はより一般的になり、治療が困難になってきている。青色光線療法は、これらの感染症を治療するための選択肢となり得る。

仮説/目的 – 本研究の目的は、メチシリン感受性黄色ブドウ球菌(MSSP)およびメチシリン耐性黄色ブドウ球菌(MRSP)に対する465 nm青色光のin vitroでの殺菌活性を測定することである。我々は、青色光の照射は、以前にメチシリン耐性黄色ブドウ球菌(MRSA)について報告されたように、in vitroで用量依存的にMSSPおよびMRSPを死滅させると仮定した。

方法 – 6回の反復実験において、各菌株 [MSSP、n = 1; MRSP ST-71(KM1381)n = 1; およびMRSA(BAA-1680)n = 1] を半固体培地上で培養し、465 nmの青色光線療法装置を用いて56.25、112.5および225 J / cm^{2}の累積投与量を照射し、35℃で一晩インキュベートした。コントロールは照射しなかった。 コロニー数(CC)は手作業で計測した。記述統計を行い、Wilcoxon-Mann-Whitney順位和検定を用いて治療効果を評価した。有意差が認められた場合、事後分析のためにボンフェローニ補正後の順位和検定を実施した。

結果 – MRSAに対してはすべての用量で青色光照射によるCCの有意な減少が認められたが(P = 0.0006)、MSSP(P = 0.131)およびMRSP(P = 0.589)については認められなかった。

結論 – 青色光線療法は、MRSAのCCを有意に減少させたが、MSSPおよびMRSPのCCは減少させなかった。MRSA分離株の相対的光感受性の機序は不明であるが、*S. pseudintermedius*と比較して、黄色ブドウ球菌中の高いポルフィリン濃度が青色光吸収の調節に起因すると仮定される。

摘要

背景 — 假中间型葡萄球菌是犬细菌性皮肤感染中最常见的病原,耐甲氧西林感染已变得越来越普遍,对其治疗也具有挑战性。蓝光光疗可能是治疗这些感染的一种选择。

假设/目的 — 本研究的目的是测定波长为465nm的蓝光,对甲氧西林敏感的假中间型葡萄球菌(MSSP)和耐甲氧西林假中间型葡萄球菌(MRSP)的体外杀菌活性。我们假设,蓝光照射会以剂量依赖的方式在体外杀死MSSP和MRSP,一如先前报道的耐甲氧西林金黄色葡萄球菌(MRSA)。

方法 — 在六组重复性实验中,将每种菌株[MSSP, n = 1; MRSP ST-71 (KM1381) n = 1;和 MRSA (BAA-1680) n = 1]在半固体培养基上培养,使用波长465nm的蓝光光疗装置,以56.25, 112.5 和 225 J/cm^{2}的累积剂量进行照射,并在35°C孵育过夜。对照组没有照射。人工执行菌落计数(CC)。进行描述性统计,并使用Wilcoxon–Mann–Whitney秩和检验评估治疗效果。当存在显著性差异时,采用Bonferroni校正秩和检验进行后续分析。

结果 — 所有剂量组的蓝光照射均能显著降低MRSA (P = 0.0006)的菌落计数量,但对MSSP(P = 0.131)或MRSP (P = 0.589)的影响不显著。

结论 — 蓝光光疗能显著降低MRSA的菌落计数量,但对MSSP或MRSP的影响不显著。目前,对MRSA菌珠相对光敏性机制尚不清楚,推测原因是由于金黄色葡萄球菌相对于假中间型葡萄球菌,含有更高的卟啉浓度,该物质能调节蓝光的吸收。

Resumo

Contexto – O *Staphylococcus pseudintermedius* é o principal causador de infecções bacterianas em cães. As infecções resistentes à meticilina vem se tornando um problema mais comum e de tratamento desafiador. A fototerapia de luz azul pode ser uma opção para o tratamento destas infecções.

Hipótese/Objetivos – O objetivo deste estudo foi mensurar a atividade bactericida *in vitro* de uma luz azul de 465 nm em *Staphylococcus pseudintermedius* suscetíveis à meticilina (MSSP) e *Staphylococcus pseudintermedius* resistentes à meticilina (MRSP). A nossa hipótese é de que a irradiação com a luz azul eliminaria MSSP e MRSP *in vitro,* em um padrão dose-dependente como foi reportado anteriormente para *Staphylococcus aureus* resistente à meticilina (MRSA).

Métodos – Em seis experimentos em duplicata, cada cepa [MSSP, *n* = 1; MRSP ST-71 (KM1381) *n* = 1; e MRSA (BAA-1680) *n* = 1] foi cultivada em meio semissólido, irradiada com luz azul de 465 nm em um aparelho de fototerapia em doses cumulativas de 56,25; 112,5 e 225 J/cm^2 e incubados durante a noite a 35°C. Os controles não foram irradiados. As contagens de colônias (CC) foram realizadas manualmente. Foi realizada estatística descritiva e os efeitos do tratamento foram avaliados utilizando o teste de Wilcoxon-Mann-Whitney rank-sum. Os testes de Bonferroni-corrigidos rank-sum foram realizados para análise post-hoc quando diferenças significativas foram identificadas.

Resultados – Houve uma redução significativa nas CC com a irradiação de luz azul em todas as doses para MRSA ($P = 0.0006$), mas não para MSSP ($P = 0.131$) ou MRSP ($P = 0.589$).

Conclusões – A fototerapia com luz azul reduziu as CC para MRSA, mas não para MSSP ou MRSP. O mecanismo para fotosensibilidade do isolado de MRSA é desconhecido, mas suspeita-se que seja devido à maior concentração de porfirina em *S. aureus,* comparado ao *S. pseudintermedius,* que modularia a absorção da luz azul.