

Visible 405 nm SLD Light Photo-Destroys Methicillin-Resistant *Staphylococcus aureus* (MRSA) In Vitro

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Background: Infections with MRSA remain a growing public health concern, prompting the need to explore alternative treatments instead of the on-going effort to develop stronger drug-based therapies. We studied the effect of 405 nm blue light on two strains of MRSA—US-300 strain of CA-MRSA and the IS853 strain of HA-MRSA—in vitro.

Methods: We cultured and plated each strain, following which bacteria colonies were irradiated with 0, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 25, 30, 35, 40, 45, 50, 55, or 60 J cm⁻² energy densities—just once—using a Solaris[®] superluminescent diode (SLD) device. Specimens were incubated at 35°C for 24 hours. Then, digital images obtained were quantified to obtain colony counts and the aggregate area occupied by bacteria colonies.

Results: Blue light irradiation produced a statistically significant dose-dependent reduction in both the number and the aggregate area of colonies formed by each bacteria strain ($P < 0.001$). Maximum eradication of the US-300 (92.1%) and the IS-853 colonies (93.5%) was achieved within 9.2 and 8.4 minutes of exposure, respectively. The longer the irradiation the more bacteria were eradicated. However, the effect was non-linear as increases of energy densities between 1.0 and 15 J cm⁻² resulted in more bacteria death than similar increases between 15 and 60 J cm⁻².

Conclusion: At low doses, blue light photo-destroys HA-MRSA and CA-MRSA in vitro; raising the prospect that phototherapy may be an effective clinical tool in the on-going effort to stem MRSA infections. *Lasers Surg. Med.* 40:734–737, 2008. © 2008 Wiley-Liss, Inc.

Key words: blue light; phototherapy; MRSA; eradication of bacteria

INTRODUCTION

Methicillin-resistant strains of *Staphylococcus aureus* (MRSA) cause serious diseases in health care and non-health care facilities [1–5]. Two billion people carry some strain of *S. aureus* world-wide; of these 53 million have MRSA, usually in their nasal cavities [1,5,6]. Treatment of *S. aureus* infections has been difficult because of widespread dissemination of plasmids containing the penicillin

cleaving enzyme penicillinase. As a result, stronger semisynthetic penicillinase-resistant penicillins, such as Methicillin, are continually developed to combat resistant isolates. However, the bacteria continue to offer resistance to each new class of antibiotics [7]. At present, less than 5% of staphylococci strains remain susceptible to penicillin, and it is estimated that 40–50% of *S. aureus* isolates are now resistant to Methicillin; a problem that continues to worsen with uncontrolled use of antibiotics [1,4,6–8].

Whereas most MRSA infections were previously acquired in hospitals, since 1980 an increasing number of cases have been reported in non-hospital community settings [1,4,5,7,8], resulting in two well-defined strains—community acquired MRSA (CA-MRSA) and hospital acquired MRSA (HA-MRSA) [1,4]. The median age for CA-MRSA infection is 23 years [2], the corresponding age for HA-MRSA clone is 68 years [1,2,8,9]. CA-MRSA has been described in urban and rural communities in persons without prior medical exposure, particularly sportsmen and sports-women [4,5,10,11]. Recent outbreaks of disease and fatalities reported in the United States were associated with CA-MRSA [10,11], the most common strains being USA-300 and USA-400 [1–3,8,12]. These strains are associated with cutaneous skin and soft tissue infections [4,8], as well as life-threatening systemic attacks [1,3,10].

The continuing resistance of MRSA to antibiotic treatment calls for alternative measures to eradicate nidi of infection and prevent the spread of contamination [1,13]. It has been shown that gram positive bacteria, such as *S. aureus*, are susceptible to photodynamic inactivation [14], particularly by UV irradiation, which is well-known to photo-destroy bacteria and other microbes [15]. Calibrating the energy fluence of UV that will destroy bacteria without damage to normal tissue is tasking and error-prone because—being inimical—photo-destruction of normal tissue may occur even with minimal overexposure to UV. Recent reports suggest that other wavelengths of the

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Accepted 2 October 2008
Published online in Wiley InterScience
(www.interscience.wiley.com).
DOI 10.1002/lsm.20724

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visible spectrum of light can photo-destroy bacteria, including *Propionibacterium acne* [16–18], and common strains of *S. aureus* and *Pseudomonas aeruginosa* [19,20]. Encouraged by these reports, we studied the effects of various doses of visible blue light ($\lambda=405$ nm) on two strains of MRSA—US-300 strain of CA-MRSA and the IS853 strain of HA-MRSA—in vitro.

METHODS

The IS853 strain was obtained from Winthrop University Medical Center, Mineola, NY, while the USA-300 strain was obtained from New York Medical College, Valhalla, NY. The identity of each strain was confirmed by standard identification procedures, including the Gram stain, hemolytic patterns on blood agar, and catalase and coagulase production. Each strain was separately diluted to a cell count of 5×10^6 /ml in 0.9% normal saline. Then, the bacteria were volumetrically streaked onto round 35 mm plates of Tryptic Soy Agar (TSA) and exposed to blue light. The applicator of the Dynatronics Solaris[®] light source was a 5.0 cm² cluster of 36 superluminescent diodes (SLDs) emitting light with 390–420 nm spectral width, 405 nm peak emission, 500 mW average power, and 100 mW cm⁻² irradiance. To ensure even irradiation of each plate, we used culture plates that were the same 5.0 cm² in size as the applicator.

The applicator was placed at a standard distance of 1–2 mm perpendicularly above each open plate to ensure direct irradiation of each bacterial culture. Plates were then exposed to 0, 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 25, 30, 35, 40, 45, 50, 55, or 60 J cm⁻² energy fluence just once before they were incubated at 35°C for 24 hours. Standard digital images of plates were taken, scanned into the computer and bacteria colonies quantified with Sigma Scan Pro 5 software. Colony counts and the aggregate area occupied by bacteria colonies were computed and used to compare the effect of fluence on both strains of bacteria. The experiment was repeated seven times with the US-300 strain and four times with the IS853 strain. Descriptive data were generated; then, ANOVA was used to determine the effect of light on both strains of bacteria.

RESULTS

As shown in Figures 1 and 2, a single exposure of each strain of MRSA to visible 405 nm light produced a statistically significant dose-dependent reduction in both the number and the aggregate area of colonies formed by the bacteria ($P < 0.001$). Maximum eradication of the US-300 (92.1%) and the IS-853 colonies (93.5%) was achieved within 9.2 and 8.4 minutes of irradiation, respectively. In general, the longer the irradiation the more bacteria were eradicated; however, the effect was non-linear. Increases of energy fluencies between 1.0 and 15 J cm⁻² resulted in more bacteria death than similar increases between 15 and 60 J cm⁻². Whereas 12 J cm⁻² fluence eradicated 50% or more of each strain of bacteria, much more fluence—55 J cm⁻²—was needed to further destroy another 40% or more of each strain (Figs. 1 and 2). Thus, doubling or

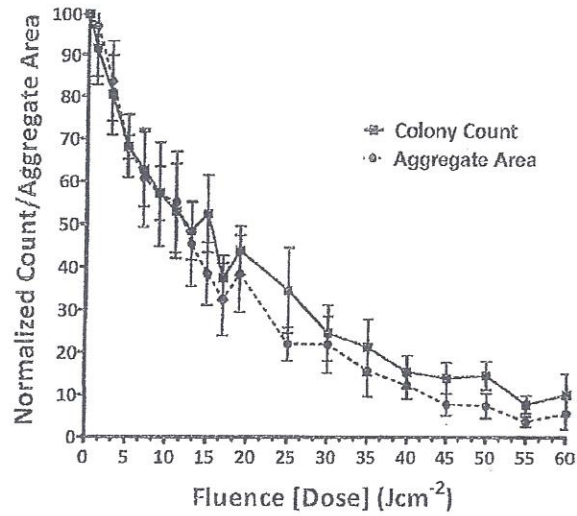


Fig. 1. Effect of 405 nm Blue Light On US-300 MRSA.

tripling the dose to 24 or 36 J cm⁻² did not double or triple the bactericidal effect; rather, doubling the dose destroyed an additional 15% of the colonies and tripling the dose further destroyed 30% more colonies.

DISCUSSION

Infections with MRSA have increased remarkably during the past decade, as efforts to find effective remedies for common strains of the bacteria remain elusive. In particular, outbreaks of CA-MRSA infections have been reported increasingly in the United States [1,11,21] and other developed nations [22]. CA-MRSA differs from the HA-MRSA in its antibiotic susceptibility profile and in the staphylococcal cassette chromosome (SCC) mec type, the

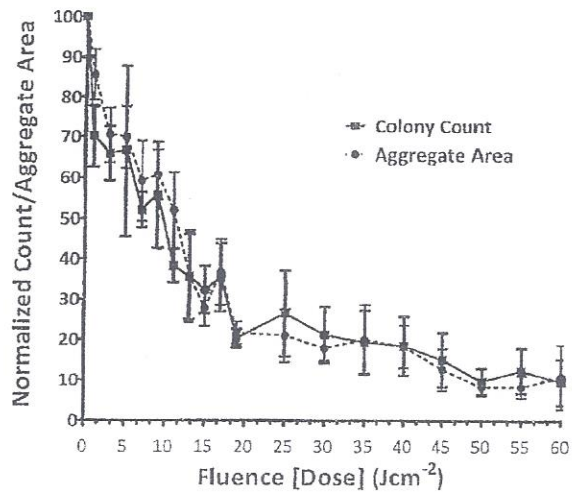


Fig. 2. Effect of 405 nm Blue Light On IS-853 MRSA.

locus for encoding Methicillin-resistance. Moreover, CA-MRSA is identified as SCC-mec type IV and is often linked with the virulence factor Panton Valentin Leukocidin (PVL); a toxin that is associated with increased risk of invasive disease as well as skin and subcutaneous tissue infection. The SCC mec A also encodes for Penicillin Binding Protein (PBP2a), which is not inhibited by beta-lactam antibiotics [23]. Consequently, available medications have had limited successes in stemming outbreaks of CA-MRSA. Even the development of new drugs, such as oral Cephalosporin, Tetracycline, Bactrim, and Erythromycin as well as the synthetic forms of penicillin, Levofloxacin, have been inadequate in limiting the spread of CA-MRSA.

The evolving genetics of CA-MRSA [23] and the mode of transmission of disease—individuals passing the isolate to their close contacts and some at risks for recurrent infection either due to their waning immune system or the aggressiveness of the bacteria—suggest the need for an alternative approach to treatment. An effective alternative treatment must remain efficacious despite the fastidious genetic variation of the bacteria and its resistance to a broad array of antibiotics. Blue light phototherapy seems to fulfill this requirement because it has the capacity to denature DNA regardless of the genetic drift of the pathogen [15,24]. The low dosages of visible 405 nm light used in our study may be a viable and valuable alternative to antibiotics, particularly in skin and subcutaneous MRSA infections that are susceptible to non-invasive irradiation. Our results show successful eradication of more than 92% of both strains of MRSA—HA-MRSA and CA-MRSA—in less than 10 minutes of treatment at relatively low irradiance and energy fluence. The irradiance and fluence used in our study fall within the range of normal light exposure experienced daily by humans, raising the prospect that phototherapy, as used in this study, could be a safe and effective clinical armamentarium in the on-going effort to eradicate MRSA, especially in topical skin and soft tissue MRSA infections. Furthermore, the effect of phototherapy on both strains of bacteria was similar; suggesting that this treatment approach can destroy MRSA regardless of its antibiotic sensitivity or genetic variation.

Our study did not explore the mechanism of photo-eradication of MRSA by blue light. However, we have reason to believe that blue light exerts similar effects on DNA as ultraviolet (UV) light. As early as 1930, Gates [15] showed that visible wavelengths of light beyond 400 nm wavelength can produce bactericidal effects similar to UV when used at higher energy densities. UV photo-destroys bacteria and other pathogens because it is absorbed in the double bond within the pyrimidine bases of DNA such as thymidine and cytosine. Absorption of UV opens the bond, allowing the UV-modified base to react with neighboring bases thereby producing either two new bonds between the neighboring bases or a single bond between two carbon atoms [15]. The resulting photo-damage of DNA can be repaired by cells. However, if the rate of damage exceeds the rate of repair, the result is cell death [15]. Perhaps this mechanism explains our findings as well as recent

reports which indicate that blue light photo-destroys *P. acne* in vitro [17] and significantly improves the clinical appearance of patients with *acne vulgaris*, when used in combination with red (660 nm light [18]).

More recently, Guffey and Wilborn [19] examined the effects of 405 and 470 nm light on two common aerobes, *S. aureus* and *P. aeruginosa*, and anaerobic *P. acnes*, in vitro. Colony counts were then used to compare their abilities to kill bacteria. Bactericidal effects were demonstrated for both wavelengths but the kill rate was higher with the 405 nm light, reaching 90% for *S. aureus* and 95.1% for *P. aeruginosa*. Neither wavelength was effective for anaerobic *P. acne*. With the superior bactericidal effect demonstrated for 405 nm light, they then examined the in vitro effect of a combination of 405 nm blue light and 880 nm infrared light on *S. aureus* and *P. aeruginosa* in their second study [20]. Colony counts again showed strong bacteria kill rates—as much as 93.8% for *P. aeruginosa* and as high as 72% for *S. aureus*. Our results are consistent with these recent reports [17–20]; moreover, they demonstrate—for the first time—that blue light photo-destroys two strains of MRSA.

Increases of energy fluencies between 1.0 and 15 J cm⁻² resulted in more bacteria death than similar increases in the dose ranges above 15 J cm⁻² (Figs. 1 and 2). This finding suggests that consecutive low doses may be more effective in combating MRSA than a single higher dose. For example, two consecutive doses of 10 J cm⁻² may be more effective than a single dose of 20 J cm⁻². Because of this finding, we are initiating studies to ascertain the effects of multiple exposure of MRSA to low dosages of blue light.

CONCLUSION

Our results warrant the conclusion that 405 nm blue light photo-destroys HA-MRSA and CA-MRSA in vitro; suggesting that a similar bactericidal effect may be achieved in vivo.

ACKNOWLEDGMENTS

This study was funded by Dynatronics Corporation, Salt Lake City, UT.

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