Effect of Diode Laser (805) nm on alpha-toxin production and antibiotic sensitivity of Staphylococcus aureus

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Abstract:
The effect of low level laser radiation on *Staphylococcus aureus* with different exposure times has been studied. Thirty skin samples [swabs] were collected from patients with burn and wound infections of Al-yarmouk teaching hospital, during the period from November 2010 to March 2011. Ten isolates of *S. aureus* were identified by their cultural characteristics, microscopic examination, and biochemical tests. The ten isolates were exposed to diode laser [805nm] at different exposure times [1min., 3min. and 5min.]. The activity of bacteria to produce alpha toxin and its sensitivity to antibiotics were determined before and after irradiation. The result of alpha toxin production of ten irradiated isolates were illustrated that toxin production was decreases after different time of exposure [1, 3 and 5 min.] of irradiation. The effect of diode laser on the sensitivity of *S. aureus* to antibiotics discs shows slightly increase in the diameter of inhibition zone to these antibiotics at different time of exposure.

Keywords: Staphylococcus,Aureus,Diod laser,light,Alpha-toxin,Antibiotic susceptibility

دراسة تأثير أشعاع الليزر ثنائي الصمام [٨٠٥] نانوميتر على إنتاجية السم ألفا وحساسية المضادات الحياتية على البكتريا Staphylococcus aureus

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الخلاصة
تم دراسة تأثير المستوى الواطئ لإشعاع الليزر ثنائي الصمام على بكتيريا المكورات العنقودية الذهبية بأوقات تعريض مختلفة [دقيقة واحدة، ثلاث دقائق، خمسة دقائق]. جمعت ثلاثين عينة جلدية من مرضى الحروق والجروح الخمجية من مستشفى اليرموك التعليمي خلال الفترة من تشرين الثاني ٢٠١٠ إلى آذار ٢٠١١ .تم تشخيص عشر عزلات من بكتريا المكورات العنقودية الذهبية من خلال المواصفات الزرعية، الفحص المجهري والاختبارات الكيموحيوية . عرضت العزلات العشرة إلى التشعيع بالليزر ثنائي الصمام [٨٠٥ نانوميتر] في أوقات تعريض مختلفة [دقيقة واحدة ،ثلاث دقائق،خمسة دقائق] .تم تحديد قابلية البكتيريا لإنتاج سم ألفا وحساسيتها للمضادات الحيوية قبل

وبعد التشعيع وقد أظهرت النتائج انخفاض إنتاج البكتيريا لسم ألفا بعد التشعيع بينما بالنسبة إلى حساسيتها للمضادات الحيوية فقد أظهرت النتائج زيادة طفيفة بالقطر التثبيطي للمضادات الحيوية المستخدمة.

Introduction:

Since the invention of lasers forty years ago, its field has been developed rapidly and the applications of lasers have been expanded from pure physics to biology, technology, chemistry, medicine and allied field [1]. The basic structure of laser consists of an amplifying medium with an inverted population between two mirrors. There are many kinds of amplifying media, which can be used in the laser to play the essential role of amplifying light through the population inversion and the stimulation emission [2].

Although little attention has been given to the bactericidal effect of laser radiation particularly using low-power lasers, it has demonstrated that He-Ne laser light has an inhibitory action on cariogenic bacteria [*Streptococcus mutans, Streptococcus sobrinus, Lactobacillus casie* and *Actinomyces viscosus*] [3]. Photodynamic therapy has been used by Lombar *et al.*(1989) to treat patients with post-surgical infections and abscesses, the bacteria involved being *Peptostreptococcus anaerobes*, *S.aureus* and *Streptococci spp.*[4]. And because of 90% of *S. aureus* strains are penicillin resistant leaving only methicillin and vancomycin to treat the majority of infections. However, with increase numbers reports of methicillin resistant *S.aureus* (MRSA) and vancomycin resistant *Entercocci* (VRE) chemists are faced with daunting task of generating antibiotics with novel modes of action, and doctors with the task of curing seemingly incurable infections[5]. Since coagulase production and Beta-hemolytic are two characteristic that link *Staphylococci* with virulence in addition to alpha-toxin which play a role in pathogenesis, we determine the effect of low-level laser radiation on *S. aureus* with different exposure times.

Materials and methods:

Isolation and identification of bacteria: Thirty skin samples [swab] were collected from patients with burn and wound infections of Al-yarmouk teaching hospital, during the period from November/2010 to March/2011. Identification had been made according to the shape, color, size, edges and height of the colony on surface of brain heart infusion (Himedia) and blood agar plates, Gram stain and API staph [Biomerieux] had been used also to identify these isolates [6]. Several tests including: Catalase test, Mannitol fermentation test, coagulase test, alpha-toxin test and Hemolysis patterns on blood agar were also used to identify these isolates [7].

Alpha hemolysin assay (Micro titer plate method)

This assay had been made to determine the presence of alpha hemolysin toxin [ten isolates] in the supernatant of *S. aureus* strains before laser exposure: Bacterial isolates were cultured in Trypton soya broth for18 hours at 37ºC. After centrifugation (7000 rpm) for 15 minutes the supernatant of each isolates transferred to sterile test tubes. Detect the ability of isolates for alpha hemolysin production by adding 0.1 ml of 2 % washed rabbit red blood cells to the well of U shaped micro titer plate (in separated plates], then 0.1 ml of bacterial supernatant, in duplicate. First and second wells of each horizontal line represent the negative control [0.1 ml of normal saline + 0.1 ml of washed red blood cells) was applied. The micro titer plate was incubated at 37 ºC for one hour. After incubation, plates were kept at 4 ºC for 2-18 hours, and then the result (lysis of RBCs) was read [8].

Antibiotic sensitivity

sensitivity of *S. aureus* isolates were performed by Kirby-Bauer disc diffusion assay [9]. The following antibiotic discs were used during this study: Ciprofloxacin (CIP (5µg)), Chloramphenicol(C(30µg)),Gentamicin(CN(10µg),Nitrofurantoin(F(300µg)), (Bioanalyse/CE).

Laser system

The CW diode laser (Eltech S.R., Italy) emitting laser light at 805 nm, IR light (light in the invisible spectrum below red from 700-2000nm) [8] and a thin flexible glass fiber with a diameter of 8mm [10], the output power of (0.94, 2.01 and 2.76) W and the exposure time was (1 min.). The power densities were 1.87, 4.0 and 5.49 W/cm2 for 0.94, 2.01 and 2.76 W, respectively.

Irradiation procedure: A loopful of the culture was transferred from the brain heart infusion agar slant to a test tube containing brain heart infusion broth and incubated at 37°C for overnight. The suspension was centrifuged at 3500 r.p.m for 10 minutes, supernatant was removed and the precipitate was resuspended using physiological saline. The suspension was mixed using vortex to get homogenous suspension, which compared with the McFarland solution ($1.5*10^8$ CFU/ml) [11]. One milliliter of the diluted bacteria suspension from each species was transferred to sterile Eppendroff tube and exposed to laser light at different exposure times, another Eppndroff tube also contain 1ml of the suspension did not exposed to laser light used as control, then irradiated and non-irradiated suspension was placed on brain heart infusion agar and incubated at 37°C for overnight, then tested for biochemical characteristics, their sensitivity to antibiotics and alpha toxin production [12].

Results & Discussion

From all isolates skin swab, ten isolates (33.3%) were identified as *S. aureus*. Results indicated that, these isolates were positive to Catalase, Coagulase, hemolysin production (alpha-toxin)and it was able to ferment Mannitol. Morphological and Biochemical characterization agreed with the data stated by [6,7]. In addition to the above tests, biochemical identification is also done by Api-staph system, which confirmed the previous conventional identification.

Treatment of ten isolates of *S.aureus* with diode laser (2w) at different exposure times, results in no effect of irradiation on the enzymes production (Catalase and Coagulase), and the fermentation of Mannitol of these isolates **(Table-1)**.

Photosensitiser of *S.aureus* isolates, using the same conditions, resulted in decrease in activity of beta- hemolysis, whereas, the results of alpha-haemolysin production shows, 40% reduction (in toxin production) at 5min.of exposure time (the results shows a significant reduction of hemolysin production in comparison to control)**(Table-1).**

Tubby *et.al*.,2009[13], found the activity of the hemolysin was completely inhibited after exposure to a light dose in the presence of methylene blue, lazer light alone had no appreciable effect on the activity and production of hemolysins of *S.aureus* isolates. So these results, showed that, alpha-haemolysin is the most susceptible of the virulence factor under test, perhaps due to the nature of its amino acid composition, which may leave it more vulnerable to attack by reactive oxygen species.

Alpha- haemolysin of *S.aureus* is a membrane damaging toxin, it is capable of lysing a number of different cell types. Alpha- hemolysin is thought to be important in infection as it has a number of detrimental effect on host cells due to the disruption of ion transport across host cell membranes, ultimately leading to apoptotic cell death and Oedema [14].

The role of alpha- hemolysin in the virulence of *S.aureus* has been demonstrated in a number of infection models such as mastitis and Pneumonia. In addition alpha- haemolysin has immunomodulatory properties, notably its ability to trigger the relase of pro- inflammatory cytokines[15]. Thus in activation of alpha-hemolysin by photodynamic treatment may protect against harmful inflammatory processes as well as eliminating infecting organisms.

The disc diffusion method was used to determine susceptibility of *S.aureus* isolates to several antibiotics (Chloramphenicol (C), Ciprofloxacin(Cip), Gentamicin (CN),and Nitrofurantion(F)). After exposure to diode laser at different times (1,3,5)min.

Results show, that change in susceptibility of most isolates to antibiotics at all different times of exposure,**(Figures-1,2,3,4)**.

(Figures-1,2)show increase susceptibility of *S.aureus* isolates to Ciprofloxacin and Nitrofurantion at one minute exposure to laser, while the same isolates show increase susceptibility to Gentamicin and Chloramphenicol at 3-5 minutes exposure to laser (there is no significant sensitivity to antibiotics) **(Figure-3,4)**.

These findings agreed with [16,17] who found that effect of diode laser increase the susceptibility of bacteria to antibiotics, with increasing time and dose of laser exposure. Whereas these results disagree with another study showed that there was no effect to the laser light on the antibiotics susceptibility of *S. aureus*[18] .

Changes in sensitivity of bacterial isolates to the antimicrobial agents after treatment with diode laser is may be due to the combination effect of laser and antimicrobial agent making the bacterial

cell more sensitive to these agents. Also these changes in sensitivity of bacteria may be due to the changing in bacterial pumping systems [efflux pump] that mainly responsible on bacterial resistance or sensitive to antibiotics. Failure of bacteria to produce specific enzymes that chemically modify specific antibiotic also maybe increased the bacterial sensitivity to the antibiotics [19,20].

Conclusion

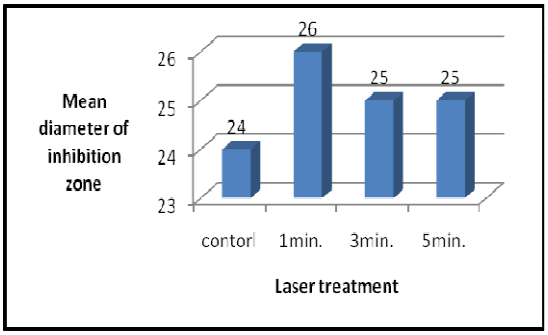
Some isolates of *S. aureus* loosed its ability to produce beta- hemolysis and alpha-toxin and increase sensitivity to antibiotics. While the ability to produce catalase, coagulase and mannitol fermentation were not affected by laser irradiation.

Recommendation:

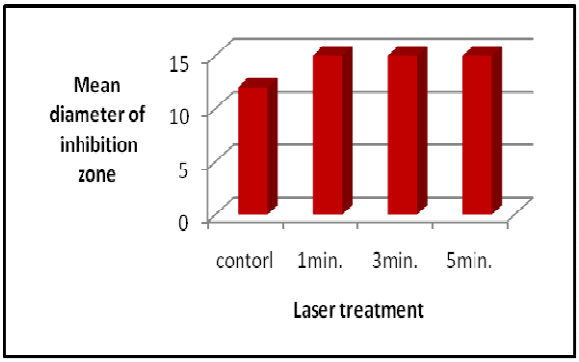
using another types of laser to study their effects on virulence factors of pathogenic bacteria for using in the treatment of infectious disease

(Table-1) Effect of Diode laser at different exposure times on biochemical tests of *S. aureus*

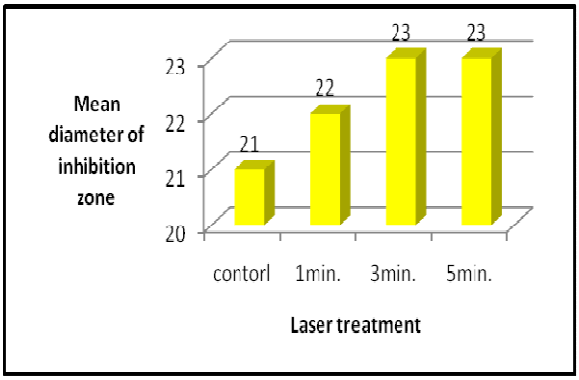
Test	Control	Laser treatment [percentage of positive test]		
		1 min	3 min	5 min
Blood haemolysis	100% [+]	45.5% [+]	18.2% [+]	63.6% [+]
Catalase	100% [+]	100% [+]	100% [+]	100% [+]
Coagulase	100% [+]	100% [+]	100% [+]	100% [+]
Mannitol fermentation	100% [+]	100% [+]	100% [+]	100% [+]
Alpha-toxin test	100% [+]	90% [+]	80% [+]	60% [+]



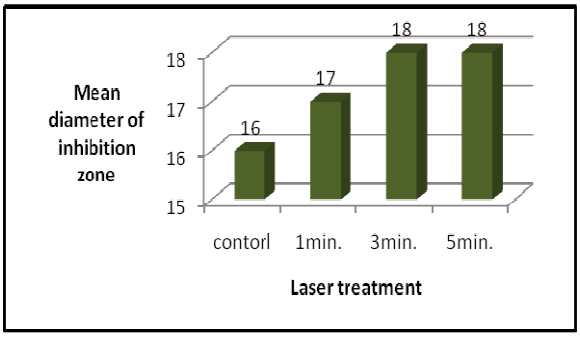
(Figure-1) Effect of exposure of *S. aureus* to Diode laser on sensitivity of Ciprofloxacin.



(Figure-2) Effect of exposure of *S. aureus* to Diode laser on sensitivity of Nitrofurantoin



(Figure-3) Effect of exposure of *S. aureus* to Diode laser on sensitivity of Gentamicin



(Figure-4) Effect of exposure of *S. aureus* to Diode laser on sensitivity of Chloramphenicol.

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