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The Effect of Low-Power Laser Combined with Providone-iodine Photosensitizer on Elastase Production of Pseudomonas Aeruginosa Isolated from Wounds

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Abstract

This study was conducted in Tikrit Teaching Hospital on 73 patient with wounds. 30 (41%) isolates of *Pseudomonas aeruginosa* were obtained from these patients. 20 isolates were randomly selected for the present study. The present study revealed a significant effect of low-power diode laser light with photochemical agent povidone-iodine (PVP-I) on elastase production and all the tested isolates were elastase-negative after exposure.

1 Introduction

Elastase is a major virulence factor in *Pseudomonas aeruginosa* that is believed to cause extensive tissue damage during infection in the human host. Elastase is secreted in non-mucoid *P. aeruginosa*. It is known that secretion of most virulence factors such as elastase, lipase, exotoxin A, etc., in *P. aeruginosa* is greatly reduced in alginate-secreting mucoid cells isolated from the lungs of cystic fibrosis (CF) patients[1].Many studies showed that the laser light alone had no significant effect on the viability of the organism. Indeed, neither the dye nor laser light alone had statistically significant effect on the viability of the organism[2,3]. Toluidine blue (TBO) used widely to sensitizer many bacteria to be killed by (He/Ne) lasers ,these bacteria were included *Staphylococcus aureus ,Pseudomonas aeruginosa ,Porphyromonas gingivalis, Fusbacterium nucleatum , Actinobacills actinomycetemcomitans,Streptococcus sanguis , Escherichia coli , Streptococcus mutans and Sarcinia lutea* [2-6].Aluminum disulphonated phothalocyanin (ADP) used to sensitize *Helicobacter pylori* to killing by copper pumped dye laser [7]. ADP also used to sensitize *Streptococcus sanguis* and *Str.mutans* to be killed by (He/Ne) gas laser [8]. Prophyrin has been used against gram-negative bacteria [9]. Povidone –

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iodine is a best known iodophore, which is a complex of elemental iodine with a carrier [9] polyvinylpyrrolidone, (pvp-I)],that provides increasing solubility of the iodine and sustained release of iodine. Povidone – iodine firstly used as photosensitizer by AL – Jebouri and AL – Obaidy in 1997[10] to sensitize *Staphylococcus aureus* to be killed by (He/Ne) laser (out put 5mW). Finally, Naoom [11] showed that photosensitizer is sensitizing laser to eradicate *Pseudomonas aeruginosa* in vitro . In fact povidone – iodine used as antiseptic agent to treat wound infection especially when a wound is infected by *P.aeruginosa* [12-14]. The present study is an attempt to assess the effect of low- power diode Laser combined with providone-iodine as a photosensitizer on elastase production of *Pseudomonas aeruginosa* isolated from the wounds.

2 Methods

Samples were taken from hospitalized patients at Saddam General Hospital in Tikrit city. Surgical wound swabs were taken on third post – operative day. Surgical and burn wounds were sampled using sterile cotton applicators moistened with 2 ml of a nutrient broth (oxoid) [15]. The samples were delivered to the laboratory within one hour of sampling where the bacteriological studies were carried out and enriched in nutrient broth at 37 ˚C for 18 hours. Each sample was subcultured on Pseudomonas selective agar (oxiod) and inocubated at 37 ˚C for 24 hours .The suspected colonies of *P.aeruginosa* were purified on nutrient agar plates. Then they were inoculated on nutrient agar slants and kept at 4˚C for farther studies [16] . The 20 purified colonies selected from different patients were further identified using the conventional methods [16,17] .

The Helium/Neon gas (He/Ne) laser with measured output of 5mW (laser Becaon , I. N.C Michigan , U.S.A) was used in the present study . This tool was emitting light with a wavelength of 650 nm in a collimated beam and diameter 1.3 mm. Povidone-iodine,10% (I.C.I.,U.K.) was used with concentration of 256 μg/ml [10].

A single colony of exposed and non – exposed isolates to laser combined with providone-iodine(PVP-I) was transferred and streaked in a rectangular shape with a width of 5 mm on nutrient agar which contained 1% elastin (sigma, U.K.) . The positive reaction was indicated by digestion of elastine granules and appearance of a clear zone around the colonies after 4 days of incubation at 37 ^{0}C and room temperature [18] .The exposure times were 30,60,120,180 and 240 seconds for each isolate tested.

3 Results

All 20 isolates of *Pseudomonas aeruginosa* from patients wounds revealed production of elastase due to degranulation of elastin.All strains exposed to laser and povidone-iodine combination became elastase – negatives . While the strains non-exposed to laser and povidone – iodine appeared elastase positive.Exposure to laser irradiation or to photosensitizer separately did not show any effect on elastase production by *Pseudomonas aeruginosa.*

Table 1: Elastase production of 20 clinical isolates of *Pseudomonas aeruginosa* before and after exposure to low-power laser and providone-iodine photosensitizer combination *+ve,positive;**,negative.

Types of isolates			
	Before exposure	After 4 days of exposure	After six months of exposure
Laser(L) -exposed	+ve *	+ve	+ve
Providone-iodine(PVP-I)-exposed	+ve	+ve	+ve
L+PVP-I- nonexposed	+ve	+ve	+ve
L+PVP-I -exposed	+ve	-ve **	-ve

It was seen that inactivation of elastase for different isolates was time- dependant of exposure,i.e 0,1,1,2 and 16 isolates lost their elastase activity at 30,60,120,180 and 240 seconds of exposure respectively.

4 Discussion

The present study showed that the strains of *Pseudomonas aeruginosa* isolated from different patients became elastase- negatives after different times of exposure to laser combined with PVP-I. Komerik et al [19] found that the proteases production of *Pseudomonas aeruginosa* was reduced significantly by irradiation with red light in the presence of toluidine blue O(TBO)in a dose dependent manner with respect to both light energy dose and the TBO concentration [20]. Povidone – iodine firstly used as photosensitizer by AL – Jebouri and AL – Obaidy in 1997[10] to sensitize *Staphylococcus aureus* to be killed by (He/Ne) laser (out put 5mW).Al-Jebouri and Al-Obaidy found that PVP-I sensitized laser for killing of *S.aureus* 4-fold than toluidine blue O(TBO). Moreover, it was clearly show that PVP-I does not only kill a wide range of bacteria but also inhibits the generation and release of bacterial exotoxins; furthermore, it also inactivates bacterial exotoxins as well as granulocyte-derived tissue-destructive enzymes and cytokines. These data support the usefulness and efficacy of PVP-I as an effective therapeutic agent to combat infection [19], it became evident that the pathophysiology and the outcome of infection are dependent on the properties of the microorganisms, e.g. synthesis of endo- and exotoxins, and on the host defense, the immune system. In addition to the microbicidal action, they studied the effects of providone-iodine (PVP-I, Betaisodona) on the generation, release and activity of exotoxins (alpha-hemolysin, phospholipase C, lipase), as well as on granulocyte-derived tissue-destructive enzymes (elastase, beta-glucuronidase) and microbial-induced cytokine

generation from human neutrophils[19].Furthermore,It was previously found that exposure of *P.aeruginosa* to series of sublethal concentrations of disinfectants might lead to enhancing antibiotic resistance[21].However,photodynamics therapy is highly worthing techniques of pathogens killing according to in vitro findings[4,10,22]
.It was suggested that NIR laser light irradiation by itself would also inhibit growth of *P. aeruginosa* in infected wounds and only the continuous-mode of irradiation was capable of killing *P. aeruginosa*[23]. However,the findings presented here are promising for utilization of lower-power laser combined with providone-iodine to kill *P. aeruginosa* causing various infections to man.

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