The lethal effects of Argon (Ar+) LASER upon Pseudomonas aeruginosa isolated from infected wounds in Tikrit teaching hospital

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Abstract

The effect of continuous Argon (Ar^+) LASER with energy of 150 mW and wave length 515 nm and density of energy (CW) 1326.964W/cm^2 upon *Pseudomonas aeruginosa* bacteria after focusing the LASER with a lens of focal length 15 cm for periods ranged between 5 – 25 minutes was studied. This study revealed that the diameters of killing areas exposed to the LASER beam were related directly to the periods of Ar^+ LASER beam application and because gausses property of LASER beam the intensity of killing was more in the center and the areas exposed to the LASER beam were distinguished into two areas; the central area was dark in color and the killing in this area was highest, almost all bacteria were killed and killing in this area was incomplete.

Introduction

In the early years after detection of LASER, a new advanced technology started to expand in using LASER beam in variable fields in industry as in metallic and plastic factory and also in medicine field where it used in different types of surgical operations in which the LASER beam proved its efficacy upon the surgical knife and this type of surgery called bloodless surgery because the heat produced by LASER leaded to formation of blood clot that bulge the blood vessels in the field of surgery [(1)].

It is well known today that the wave length of the LASER ray occupy the area from the ultraviolet (UV) area which wave length reach 1000 Å to the infrared area including the visible light area wave lengths and the levels of energy range from microwatt (mW) to many megawatts (MW) according to the type and wave length of LASER [(2)].

The most important applications of LASER are the using of N_2 LASER as a cellular LASER knife due to its high accuracy; it is useful in inducing genetic mutations because LASER can cut the chromosomes in addition to produce cut in one side of the cell [(3)]. It is also useful for hasten the healing after tooth extraction due to diminish the chance of getting bacterial infection by using multisections of low energy LASER for 10 minutes period [(4)]. LASER also useful in the management of tumors, this is because of the lethal property of LASER upon certain cells [(5)]. Also the using of Ar^+ LASER as a sensitive knife in surgical operations and its usefulness in the management of peptic ulcer and it is useful also in the management of congenital hemangiomas. Researches have been proved that if we can deliver the LASER ray to the sites of thrombus via the fiber-optics it leads to evaporation of the thrombus and make the arteries patent again [(1)]. Higher energy LASER(10^6 W/m) is used which has no obvious thermal effect, this also affect the bacteria by causing release of free electrons from ionization of atoms and molecules and spread inside the bacteria causing bombing waves this mechanism is important also for the destruction of calcified thrombi and small stones which does not affected by evaporation[(6)].

Pseudomonas aeruginosa is an important cause of wound infection beside *E. coli*, *Staphylococcus aureus* and *Streptococcus* spp.[(7)]. *Pseudomonas*

aeruginosa is a unipolar bacteria that inhabit water, soil, walls, furniture, air ducts and hospital food in many instances it is 0.5 – 0.8 μm[8]. This bacteria is becoming more virulent and more resistant to antibiotics, genus *Pseudomonas* composed of more than 140 species, most of them are saprophytic and more than 25 species are attached to human and *Pseudomonas aeruginosa* is the most familiar to the man[9].

The effect of different LASER rays on the living materials depends in general on the wave length, the energy of LASER and the period of exposure in addition to way of using LASER as pulses or continuous beam, bacteria affected by these factors suffered from many changes in the physiological state especially the thermal conduction and pH[10]. Regarding the living materials like bacteria some of them are chromophil due to the presence of active materials called chromatoforte which has the ability to absorb rays of certain wave lengths, these active materials contain unsaturated bonds like C=C, C=O and N=N, during the absorption the energy of photons transferred to the bacteria leading to increase the temperature by breaking the bonds so the photon energy changed into thermal energy [11].

This study aimed to investigate the lethal effect of Ar^+ LASER on the *Pseudomonas aeruginosa*.

Materials and Methods

This study include 15 patients in Tikrit teaching hospital who suffered from infected wounds and burns within the period from September to November 2007 and the equipments and materials used were blood agar, MacConkey agar, tryptophan broth, Kovac's reagent, glucose peptone water, methyl red reagent, 40% potassium hydroxide, 5% solution of alpha-naphthol, Simmon's citrate medium, incubator, autoclave, electric hot plate, light microscope and Ar^+ LASER device with lens.

Swabs from patient's wounds and burns were collected under aseptic technique and culture done on the blood and MacConkey agars. The plates were incubated at 37ºC for 24 hours. Diagnosis of bacteria is done by the identification of morphology of colony. While the growth requirements, Gram stain and other conventional methods like imvic. To irradiate the diagnosed bacteria, the bacterial colony top touched by a loop and inoculate these bacteria on blood agar by spread them over the surface in three directions at 60º and incubate the plates at 37ºC for 24 h.

Focusing of LASER ray: LASER emission apparatus produce unidirectional light of high temperature, this beam of light characterized by small diverging angle, which is the angle between the edge and the axis of the beam. Focusing of LASER ray is possible to a very small location with high accuracy, the energy which carried by focused LASER beam on a small area for e.g. by using a lens with focus length = f, to focus LASER beam with diverging angle = A, the diameter of area exposed to the LASER(S) = $f\theta$ [1].

If the LASER energy was W then the density of energy for a surface area unit (ξ) was $W/m^2 = 4W/ T\theta f^2$. The diverging angle of Ar^+ LASER was already known = 0.8º [12].

Irradiation of the 15 bacterial samples by using focused beam of Ar^+ LASER by a lens with focus length 15 cm and at a distance of 15 cm from the lens and for a periods of 5, 10, 15, 20 and 25 minutes. In this study we used Ar^+ LASER of wave length 515nm, energy 150 mW and density of energy 1326.964 W/cm^2.

Results

The present study revealed that using focused Ar^+ LASER of wave length 515nm and energy 150mW with using convex lens of focal length 15cm to irradiate bacterial colonies leaded to formation of two distinguished areas in the bacterial colony depending on the degree of killing due to gausses distribution of energy of Ar^+ LASER ray, central dark area (R_1) in which the killing was complete and peripheral light color area (R_2) where the killing was incomplete. The diameters of both areas were related directly with length of periods of exposures to the LASER ray as shown in table 1 where the diameters of R_1 ranged $(7.35 – 1.65)10^{-2}$mm for periods of exposure (5 - 25) minutes and also table 2 shows that R_2 diameters ranged $(16.25 – 3.65)10^{-2}$mm for periods of exposure (5 – 25) minutes.

Discussion

The studied bacteria which exposed to Ar^+ LASER radiation directly without using disinfectant or antibiotics or thermal treatment and because of gausses pattern in distribution of energy of Ar^+ LASER rays i.e the intensity of radiation was highest in the center and lowest in the periphery, this property lead to distinguish the areas exposed to LASER ray into two areas; the central dark area of complete killing and the peripheral light color area of incomplete killing. The diameters of areas of killing (the complete and partial) were related directly with the periods of exposure of bacteria to LASER radiation, if the diameter of irradiated area for period of 5 minutes with that of 10 minutes was compared, the increment in the diameter was not very clear because of unavailability of highly sensitive camera but the difference with the diameters of area of exposure to LASER of periods 15, 20 and 25 minutes were clear because the killing areas were relatively large compared to the former areas.

The killing of bacteria in this area was due to the absorption of LASER rays by the bacteria and transformation of light energy into heat energy in the first degree, the intruding LASER rays into the bacterial cell causing increase in the energy of motility of molecules in addition to the increment in temperature of the cell led to breakdown of cell wall and leakage of living material to the outside, so the longer the exposure time to the LASER rays the more the bacteria will killed and this result did agree with the previous results [13, 14, 15, 16, 17, 18, 19, 20, 21 and 22]. Also the complete killing of bacteria was achieved by inhibition of vital reactions inside the cell when the production of energy necessary for vital processes was inhibited.

The high intensity of LASER rays causes photo oxidation in the presence of biological stains leads to production of effective free radicals like O^{-1} which leads to destruction of cell wall and impairs the permeability of cell wall and denaturation of the protein and loss of enzymes activities, this result agreed with previous results [2, 3, 6, 15, 16 and 18].

While the partial killing was due to the penetration of LASER rays into the bacterial colony in an accumulative pattern, during this process LASER rays absorbed by the bacteria led to increase their temperature and finally death of bacteria, the remaining temperature scattered to the neighboring colonies leaded to partial killing area formation and this result agreed with the previous results [13, 14, 15, 16 and 18]. From the above results and discussion, it was found that there was a direct relationship between the diameter of killing areas (complete and partial) and the periods of exposure of bacterial colonies to the LASER rays as shown in tables 1 and 2.

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Table (1) The diameters of dark areas of complete killing R_1 and exposure periods to LASER ray.

R_1 diameters x 10^{-2}mm	**Time in minutes**
7.35	5
6.45	10
4.7	15
3.15	20
1.65	25

Table (2) The diameters of light areas of incomplete killing R_2 & exposure periods to LASER ray.

R_2 diameters x 10^{-2}mm	**Time in minutes**
16.25	5
12.8	10
9.6	15
6.7	20
3.65	25