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Bactericidal Properties of a Defocused Nd-YAG-Laser Beam

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Nd-YAG laser radiation of 1.06 or 1.32 μ wavelength killed a proportion of *Staphylococcus aureus* and *Escherichia coli* cells at power densities of 40 to 80 W/cm^2 and effected a 100% kill of these cells at a power density of 1000 W/cm^2. In blood agar, the radiation of 1.06 μ produced bactericidal effects to a depth twice that of the radiation of 1.32 μ. Laser radiation of these wavelengths did not cause elimination of R plasmids and had no effect on the antibiotic resistance of bacteria.

Key Words: *Nd-Yag laser; bactericidal properties; antibiotic resistance of microorganisms*

The conventional methods used to control wound infection, which rely on antibiotics (or other antimicrobials), are not always effective because of the increased resistance developed by bacteria and fungi to most of the current antibiotics and also because these drugs do not penetrate deep enough into inflamed tissues, especially when applied topically. Moreover, antibiotics tend to sensitize the host organism and lower immunity [6,10]. Hence the importance of the search for new chemical and physical agents with antimicrobial activity.

Recent years have seen a steady increase in the use of lasers in medicine. Research into various aspects of their interaction with living organisms has shown that low-energy radiations, in particular those of helium-neon lasers, which are now more popular than other types, either have no effect on microflora or even stimulate bacterial growth [5,9]. By contrast, high-energy lasers exert bactericidal effects at the large power densities (4000-8000 W/cm^2) used to cut or vaporize tissues.

The mechanism of the bactericidal effects produced by lasers is not straightforward. At or near the tissue surface, bacterial death is evidently caused by heat which destroys products of protein nature, but it has not been ascertained how such effects occur deep inside tissues or when wounds are treated with a defocused laser beam of relatively low power [7].

Unlike the radiation from carbon-dioxide lasers, whose energy is retained almost completely in the superficial cell layers, the radiation of wavelength λ=1.06 μ from a neodymium-doped yttrium-aluminum-garnet (Nd-YAG) laser is only slightly absorbed by water and blood and penetrates deep into the tissue, where it releases its energy in dense structures [4,8]. The latter property may prove particularly useful in the control of wound infections involving the spread of microorganisms deep inside viable tissues [3]. The radiation of wavelength 1.32 μ is less penetrating; in physiological saline, for example, its absorption was found to be 10 times greater than that of 1.06 μ radiation [1].

In the present study, we evaluated 1) bacterial survival at the surface of and deep inside a nutrient medium exposed to Nd-YAG laser radiation of 1.06 or 1.32 μ at different power densities and 2) the influence of these radiations on the antibiotic resistance of bacteria containing R plasmids. These plas-

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mids carry genetic information coding for resistance transfer factors (which confer antibiotic resistance on the bacteria) and may be wiped out by the action of a chemical or physical agent.

MATERIALS AND METHODS

We used a Nd-YAG laser operating in a pulse mode at wavelengths of 1.06 and 1.32 μ and having a peak output power of 35 W for 1.06 μ and 20 W for 1.32 μ.

Individual colonies from 20-hour cultures of *Staphylococcus aureus* 209 grown on meat infusion blood agar (MIA) and *Escherichia coli* J53 Nal^R met-63 prozz RP4 (bacteria of the Ap, Tc, Km phenotype carrying the R plasmid RP4 conferring resistance to ampicillin [Ap], tetracycline [Tc], and kanamycin [Km]) grown on Endo medium were irradiated by the laser with power densities of 40 to 4400 W/cm^2, each colony being exposed to 5 or 6 consecutive pulse series of 1-sec duration each. Unexposed colonies served as controls.

After the exposure, colonies were cut out together with pieces of the nutrient medium, placed in Eppendorf tubes containing 0.9% NaCl solution, and homogenized in a Vortex mixer, after which their serial 10-fold dilutions (0.2 ml) were plated onto dishes with the MIA (for *S. aureus*) or Endo medium (for *E. coli*) and, 20 h later, the grown colonies were enumerated to determine the number of colony-forming units (CFU).

TABLE 1. Bactericidal Effects of Radiation from the Nd-YAG Laser at Different Depths of the Nutrient Medium

Agar layer thickness, mm	CFU, ×10^5	
	λ=1.06 μ	λ=1.32 μ
Control	72±8	72±8
1-2	0*	0*
3-4	0*	1±1*
5-6	4±1*	7±1*
7-8	5±1*	14±3*
9-10	10±3*	20±2*
11-12	22±4*	28±4*
13-14	38±3*	40±3*
15-16	42±4*	62±3
17-18	39±5*	68±9
19-20	59±4*	72±6
21-22	54±4*	74±3
23-24	52±3*	70±8
25-26	53±5*	71±4
27-28	69±4	80±6
29-30	71±6	72±7

Note. *$p<0.05$ relative to the control group.

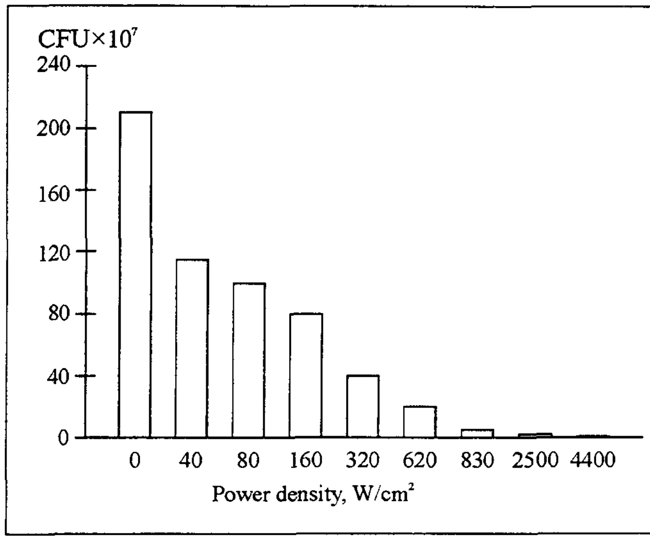

Fig. 1. Bactericidal effects from the Nd–YAG laser as a function of its radiation power density. All results were significant at $p<0.05$.

To estimate the bactericidal effects from the laser at different depths of the culture medium, an *S. aureus* broth culture grown to the stationary phase was added to and thoroughly mixed with a blood agar melted and cooled to 42-45°C and the mixture was dispensed into plastic tubes with an inner diameter of 2 mm. After solidification, the *S. aureus*-containing nutrient medium was irradiated, perpendicular to its surface, with 20 laser pulse series of 1 sec each at a power density of 4400 W/cm^2. The agar column was then cut off in layers together with the tube so as to obtain cylinders 2 mm high; after the treatment of these as described above, the number of CFU was determined and compared with their number in the control cylinders prepared from unexposed tubes.

The impact of laser radiation on bacterial antibiotic resistance was evaluated by comparing the phenotype of bacteria exposed to sublethal doses with their initial (pre-exposure) phenotype; to this end, bacteria were replica-plated onto MIA dishes with serial antibiotic dilutions [2].

RESULTS

Bactericidal effects of radiation from the Nd-YAG laser at different power densities are shown in Fig. 1. Significant decreases in the number of CFU were already noted after the 20-h blood agar-grown *S. aureus* colonies or Endo medium-grown *E. coli* colonies were irradiated at 40-80 W/cm^2. Irradiation with a power density of 1000 W/cm^2 or more resulted in a virtually complete (100%) sterilization of the nutrient medium surface. No appreciable difference was observed between the bactericidal effects of radiation at 1.06 and 1.32 μ.

In contrast, the depths at which bactericidal effects were evident strongly depended on the radiation wavelength (Table 1). After the irradiation at 1.06 μ, a significant fall in the number of *S. aureus* colonies per unit agar volume was noted at depths of 25-26 mm *vs.* only 13-14 mm after the irradiation at 1.32 μ.

The evaluation of laser radiation for its influence on the antibiotic resistance of bacteria carrying antibiotic resistance plasmids (R plasmids) indicated that exposure to sublethal doses of either wavelength (1.06 or 1.32 μ) did not alter their antibiotic resistance phenotype.

To sum up, radiation of wavelengths 1.06 and 1.32 μ from the Nd-YAG laser exerted bactericidal effects at power densities as low as 40-80 W/cm^2 and led to a practically complete sterilization at a power density of 1000 W/cm^2; after the exposure to radiation of 1.06 μ, the depth at which bactericidal effects were apparent in blood agar was twice that after the exposure to radiation of 1.32 μ; finally, radiation of either 1.06 or 1.32 μ failed to eliminate R plasmids and did not affect the antibiotic resistance of the bacteria.

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