ORIGINAL ARTICLE

Comparative Study of Argon, Nd: YAG and CO₂ Lasers to Achieve Similar Histological Changes in ddY Mouse Skin

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Abstract

The argon, Nd: YAG and CO₂ lasers have been mostly used in clinical medicine. Their histological changes are wavelength dependent and manifest their characteristics according to their irradiation time. Although their early skin changes appear clinically similar, there are no exact hard data to correlate their irradiation time. This study was designed to compare the irradiation time of these 3 lasers, using positive Evans blue exudation test (EBE) from the irradiated vascular walls as an indicator. The dorsum skin of the ddY mouse, 12 in total for each laser group, was irradiated at 3 watts of output with a spot size of 2.0 mm in diameter. The EBE appeared at 0.4 seconds for the argon laser, 19 seconds for the Nd: YAG laser and 0.04 seconds for the CO₂ laser, respectively. The significant difference for each laser system was demonstrated as follows: argon laser between 0.3 and 0.4 seconds ($p < 0.01$), Nd: YAG laser between 18 and 19 seconds ($p < 0.01$) and CO₂ laser between 0.03 and 0.04 seconds ($p < 0.01$). Histologically, all 3 lasers showed a similar homogeneous eosinophilic change of the upper dermal collagen fibers, despite the different exposure time. In conclusion, the comparison of the irradiation time for each laser with argon standardized at 1 was 47.5 for the Nd: YAG and 0.1 of the CO₂. It is suggested that these correlation might be clinically useful in the medical application of these 3 lasers.

Key words: ddY mouse, Evans blue exudation, power density, energy density

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Introduction

The 3 lasers most commonly used in medicine and surgery are the visible blue/green argon ion laser (wavelength = 488,514.5 nm), the invisible near infrared neodymium YAG (Nd: YAG) laser (wavelength = 1,064 nm) and the invisible far infrared carbon dioxide (CO₂) laser (wavelength = 10,600 nm). Their histological changes are wavelength dependent¹ and manifest their characteristics according to their irradiation time.^{2,3} Although their early skin changes appear clinically similar, there are no exact hard data to correlate their irradiation time. This study was designed to detect the correlation among these 3 lasers in the dorsum skin of the ddY mouse, with constant output power and irradiated area, using Evans blue exudation from the irradiated vascular wall (EBE),^{4,5} as an indicator.

Materials and Methods

The argon system used was a Lexell 295 argon ion system giving a visible beam, the near infrared energy was supplied by a Control 512 Nd: YAG system, and the far infrared energy from a Nippon Infrared Industries Company (NIIC) Lasery 30 Z CO₂ Laser system. The argon and Nd: YAG systems both used a flexible fiberoptic light guide to deliver the laser energy to the target tissue, terminating in a handpiece with a 10 mm focal length lens. The CO₂ system used a mirrored articulated arm, terminating in a handpiece with a 30 mm focal length lens. As both the CO₂ and Nd: YAG laser beams are in the invisible infrared, a visible helium neon (HeNe) laser was used as the aiming beam, and spot size indicator.

These 3 kinds of lasers used a diverging defocused beam with a spot size of 2 mm in diameter. The spot size was maintained by means of a distancing probe (Fig. 1).

As indicator of the safety standard, Evans blue exudation from the affected vascular walls^{4,5} was used, because Evans blue particle is very small (0.5–0.6 nm) and constant in size, and after passing out through the vessel it becomes macroscopically visible through the overlying dermal and epidermal tissue at a certain and irreversible stage in the overall photobiologic effect.⁶

Six white male ddY mice, weighing 25 g, were used as one group to each laser. The hair was shaved to a length of 0.7 mm. Two ml of 1% Evans blue saline solution (Merck Co., Ltd.) was injected into each subject intraperitoneally. The subjects were anaesthetised using ether.

Five exposures were duplicated on both lateral dorsa of each mouse, with an output power of 3 W, and a spot size of 2.0 mm in diameter using a distancing probe. At 40 minutes after irradiation, the irradiated sites were checked for the EBE, and the doses were noted to achieve the EBE for each laser system. From these doses the energy densities were calculated. The irradiated areas of the experimental animals were pro-

Fig. 1 Distancing probe: note Velcro strap to attach probe to handpiece, and measuring marks, (5.0 mm gradations), to position handpiece on probe.

cessed routinely and the specimens were evaluated histologically with hematoxylin eosin, van Gieson's and Mallory's staining, respectively, and the change of the irradiated skin was recorded by 3 layers according to the depth. The data were tested statistically using χ^2 and U tests, with the significant difference set at a p value less than 0.05.

Results

Tables 1, 2 and 3 show the macroscopic evaluation of the EBE for the argon, Nd: YAG and CO₂ lasers, respectively (Figs. 2, 3, 4), where the minus sign represents negative, and the plus sign positive reactions. The EBE appeared at 0.4 s for the argon laser, 19.0 s for the Nd: YAG laser, and 0.04 s for the CO₂ laser. The significant difference for each laser system was demonstrated as follows: argon laser between 0.3 s and 0.4 s ($p < 0.01$), Nd: YAG laser between 18 s and 19 s ($p < 0.01$) and CO₂ laser between 0.03 s and 0.04 s ($p < 0.01$).

Histologically, for the argon laser at an exposure time of 0.2 s, eosinophilic change appears in the epidermis; a 0.3 s, this reaction increased in the epidermis accompanied by a swelling of superficial dermal collagen fibres. At 0.4 s epidermal disruption occurred. Eleven out of 12 subjects accompanied by a homogeneous change in the upper dermal collagen fibres and 1 out of 12 subjects demonstrated the mid dermal damage

*Table 1 Macroscopic Evaluation of Appearance of
Evans Blue Exudation by Argon Laser*

Irradiation time (sec)	—	+	χ^2 test
0.2	12	0	>10.7 [p<0.01]
0.3	10	2	
0.4	1	11	
0.5	0	12	
0.6	0	12	

*Table 2 Macroscopic Evaluation of Appearance of
Evans Blue Exudation by Nd: YAG Laser*

Irradiation time (sec)	—	+	χ^2 test
17	12	0	>8.2 [p<0.01]
18	10	2	
19	2	10	
20	0	12	
21	0	12	

*Table 3 Macroscopic Evaluation of Appearance of
Evans Blue Exudation by CO₂ Laser*

Irradiation time (sec)	—	+	χ^2 test
0.03	10	2	>8.2 [p<0.01]
0.04	2	10	
0.05	0	12	
0.06	0	12	
0.07	0	12	

Table 4 Histological Evaluation of Tissue Damage Depth by Argon Laser

Irradiation time (sec)	Epidermis	Upper dermis	Mid dermis	U test
0.2	11	1	0	>3.08 [p<0.01]
0.3	9	3	0	
0.4	0	11	1	
0.5	0	7	5	
0.6	0	1	11	

Table 5 Histological Evaluation of Tissue Damage Depth by Nd: YAG Laser

Irradiation time (sec)	Epidermis	Upper dermis	Mid dermis	U test
17	11	1	0	>2.84 [p<0.01]
18	9	3	0	
19	2	9	1	
20	0	4	8	
21	0	0	12	

Table 6 Histological Evaluation of Tissue Damage Depth by CO₂ Laser

Irradiation time (sec)	Epidermis	Upper dermis	Mid dermis	U test
0.03	12	0	0	>3.4 [p<0.001]
0.04	4	8	0	
0.05	0	12	0	
0.06	0	4	8	
0.07	0	0	12

Fig. 2 Evans blue exudation of argon laser in ddY mouse skin at 3 W, 2 mm diam. and the irradiation time as follows:

A, 0.2 s, (-); B, 0.3 s, (-); C, 0.4 s, (+); D, 0.5 s, (+); E, 0.6 s, (+).

Fig. 3 Evans blue exudation of Nd:YAG laser in ddY mouse skin at 3 W, 2 mm diam. and the irradiation time as follows:
A, 17 s, (-); B, 18 s, (-); C, 19 s, (+); D, 20 s, (+); E, 21 s, (+).

Fig. 4 Evans blue exudation of CO₂ laser in ddY mouse skin at 3 W, 2 mm diam. and the irradiation time as follows:
A, 0.03 s, (-); B, 0.4 s, (+); C, 0.05 s, (+); D, 0.06 s, (+); E, 0.07 s, (+).

Fig. 5a ddY mouse skin, argon laser at 3 W, 0.4 s, spot size 2.0 mm in diameter: localised homogenisation in the upper dermis. Irradiated area extends from arrow at left off right side of the print. (HE stain, $\times 100$)

(Fig. 5 and Table 4). At 0.5 s, this homogeneous change spread to the mid-dermal collagen. At 0.6 s, the mid-dermal homogeneous change spread to peripheral regions.

Histological findings for the Nd: YAG laser were as follows: at 17 s an eosinophilic change was noted in the epidermis. At 18 s, this was more marked, with swelling of the superficial dermal collagen fibres. In 3 out of the 12 specimens, a homogeneous change in the superficial collagen fibres appeared. At 19 s in 9 out of 12 cases this homogeneous change was noted (Fig. 6 and Table 5). In 1 case, this change spread to the mid-dermis. At 20 s, the homogeneous change in the mid-dermis increased. At 21 s, this change spread peripherally in the mid-dermis.

For the CO₂ laser, histological findings were as follows: at 0.03 s, detachment of the stratum corneum from the other epidermal layers occurred, and slight eosinophilic change appeared in the remaining epidermis. At 0.04 s, 8 out of 12 subjects demonstrated this eosinophilic change in the superficial dermal fibres, at the same time, swelling and a homogeneous change occurred in the superficial layers of the dermis (Fig. 7 and Table 6). At 0.05 s, destruction of the epidermis occurred in all cases accompanied by homogeneous change in the superficial dermal collagen, and at 0.06 s, this change penetrated to the mid-dermis and spread to the peripheral region. Significant differences can be seen to the histological evaluation of tissue damage depth of 3 lasers

Fig. 5b ddY mouse skin, argon laser at same parameters: increased pyknosis of epidermal and superficial dermal cell nuclei with intense eosinophilia of epidermal cell protoplasm. Homogeneous eosinophilic change of the upper dermis also seen. (H-E stain, $\times 400$)

(Tables 4, 5, 6).

Discussion

The passive cutaneous anaphylactic reaction is a term given to the immediate allergic response in dermal tissue of the sensitized animal.⁷ When the exogenous stimulation to the capillary walls of the non-sensitized animal is thermal in nature as a result of a photobiologic reaction following laser irradiation of skin, some pyknotic changes occur in the vascular walls, as a result, Evans blue exudation occurs. From the histological data, the EBE occurred for all lasers used when the homogeneous change and swelling was noted in the superficial dermis.

When the permeability of the blood vessel walls is affected, progressively larger particles are allowed to pass through them. Evans blue particles (0.5–0.6 nm) will pass

Fig. 6a ddY mouse skin, Nd: YAG laser at 3 W, 19 s, spot size 2.0 mm in diameter: widespread localised homogeneity of upper dermal collagen. Irradiated area extends from arrow at right off left side of the print. (H-E stain, $\times 100$)

through the superficial dermal capillary wall at a certain and repeatable stage of the photothermal reaction. The macroscopic appearance of a blue stain in the treated area, the EBE, will therefore indicate that the reaction is occurring. Thus dosimetry to elicit the EBE, will be certain and repeatable for genetically similar biologic tissue.

The exposure times to achieve the EBE were 0.4 s for the argon, 19 s for the Nd: YAG and 0.04 s for the CO_2 laser. This means that the argon requires 10 times longer than the CO_2 to achieve the same effect, and the Nd: YAG requires 475 times longer than the CO_2 to achieve the same reaction.

Basic laser/tissue reactions are wavelength dependent¹ and mostly photobiologic in nature.^{2,3} The main wavelengths at which the argon laser operates are 488 nm and 514.5 nm. Erythrocytes absorb heavily at this wavelength. The radiant heat energy occurs first in the erythrocytes in the bloodstream, then spreads to the endothelial cells of the blood vessel wall in milliseconds. Thus this laser is used for the treatment of vascular anomaly nevi such as hemangioma simplex or port wine stain. The energy density required to achieve the EBE with the argon laser was

$$\frac{3 \text{ W} \times 0.4 \text{ s}}{\pi \times (0.1 \text{ cm})^2} = 38.2 \text{ J/cm}^2$$

Fig. 6b Area on right of Fig. 6a at higher magnification: normal architecture at right gradually displaying increased pyknosis of epidermal and upper dermal nuclei, with intense eosinophilia of epidermal cell protoplasm. Homogeneous eosinophilic change in superficial dermis at upper left dermis. (H-E stain, $\times 400$)

The wavelength of the Nd: YAG laser is 1,064 nm in the near infrared. At this wavelength the prime absorber is protein, and there is no pigment specificity, so therefore the penetration and forward scattering of this laser wavelength are great.¹

The energy density required to achieve the EBE with the Nd: YAG laser was

$$\frac{3 \text{ W} \times 19 \text{ s}}{\pi \times (0.1 \text{ cm})^2} = 1814.13 \text{ J/cm}^2$$

The CO₂ laser operates in the far infrared at 10,600 nm. This wavelength is almost totally absorbed in water molecules.⁸ The human body is composed on average of approximately 70% water. All cells are rich in water so there is no particular tissue specificity for the CO₂ laser. The fact that it needs less power to achieve the same effect as the argon and Nd: YAG lasers means it can be used in surgery as an efficient photo-vaporiser to debulk or incise tissue.⁹ The energy density needed to achieve the EBE is

Fig. 7a ddY mouse skin, CO₂ laser at 3 W, 0.04 s, spot size 2.0 mm in diameter: localised homogeneous change in upper dermal tissue. Irradiated are extends from arrow at left off right side of the print. (H-E stain, $\times 100$)

calculated for the CO₂ laser as follows:

$$\frac{3 \text{ W} \times 0.04 \text{ s}}{\pi \times (0.1 \text{ cm})^2} = 3.82 \text{ J/cm}^2$$

The argon laser beam is pigment specific, and so passes through the epidermis, superficial dermis, and blood vessel walls to be absorbed in the erythrocytes in the superficial dermal capillaries.^{10,11} In passing through the upper tissues, the beam is reflected, refracted and diffused. Thus greater energy than the CO₂ is required to achieve the EBE. Accordingly, the EBE of the argon laser should be less than the CO₂ laser, but due to the reflective/refractive effect of the epidermis, hairs and hair follicles, the value of the EBE for the argon laser was 10 times that of the CO₂ at 38.2 J/cm².

The YAG laser has no pigment specificity, and no real absorbing medium except protein. The energy transformation from light to heat still occurs, but in a slower and more gentle manner than the CO₂ or argon lasers. Because of the lack of tissue and pigment specificity at this wavelength, the beam penetrates and scatters much further into the tissue than the CO₂ and argon lasers. Accordingly the volume of tissue irradiated is much greater. It takes less energy to heat 1 ml of water to a specific temperature than to heat 1 litre of water to the same temperature. This explains the large difference in

Fig. 7b Area indicated on left of Fig. 7a at higher magnification: Pyknotic change of epidermal and upper dermal tissue seen gradually appearing from normal architecture at left of print with intense eosinophilia of epidermal cell protoplasm. Homogeneous eosinophilic change of upper dermal tissue also observed in same areas as pyknosis of the cell nuclei. (H-E stain, $\times 400$)

the value of the Nd: YAG EBE.

This vast difference between the CO₂ and Nd: YAG induced EBE values seems great. The difference is explained by the different absorption mechanisms of the wavelengths.⁹ The CO₂ laser beam is primarily absorbed in water, therefore affects the epidermis and superficial dermal tissue almost instantaneously.

The argon laser effect in tissue is less precise, and is not limited to the immediate target area due to the spread of heat from the target tissue to surrounding tissue. The argon laser is mostly used to photocoagulate skin lesions, where this spread is advantageous, rather than a disadvantage.

The YAG laser effect in tissue is widespread and imprecise. It is mostly used where deep photocoagulation of tissue is required, without real precision. By altering the power density of any one of these lasers, however, different effects can be achieved. With identical power densities, as demonstrated in ddY mouse skin, the exposure times

to achieve the same effect, the EBE, were greatly different for the reasons outlined above.

The CO₂ laser effect to the tissue is certain. Most users for the CO₂ laser in surgery involve certain vaporisation to debulk tissue, or extremely precise linear vaporisation to excise or incise tissue.

The fact that the EBE occurred concomitantly with homogeneous eosinophilic changes in the upper-dermal collagen fibres were similar for all 3 lasers, and the correlation of the irradiation time for each laser with argon standardized at 1 was 47.5 for the Nd: YAG and 0.1 for the CO₂. It is suggested that these comparison might be clinically useful in the medical application of these lasers.

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