Effect of Wavelength on Cutaneous Pigment Using Pulsed Irradiation

K. A. Sherwood, M.D., S. Murray, M.Sc., A. K. Kurban, M.D., and O. T. Tan, M.D.
Department of Dermatology, Boston University School of Medicine and The Laser Center at Boston University Medical Center Boston (KS, AKK, OTT) and Candela Laser Corporation, Wayland, Massachusetts, U.S.A. (SM)

Several reports have been published over the last two decades describing the successful removal of benign cutaneous pigmented lesions such as lentigines, café au lait macules' nevi, nevus of Ota, and lentigo maligna by a variety of lasers such as the excimer (351 nm), argon (488, 514 nm), ruby (694 nm), Nd:YAG (1060 nm), and CO_2 (10,600 nm). Laser treatment has been applied to lesions with a range of pigment depths from superficial lentigines in the epidermis to the nevus of Ota in the reticular dermis. Widely divergent laser parameters of wavelength, pulse duration, energy density, and spotsizes have been used, but the laser parameters used to treat this range of lesions have been arbitrary, with little effort focused on defining optimal laser parameters for removal of each type. In this study, miniature black pig skin was exposed to five wavelengths (504, 590, 694, 720, and 750 nm) covering the absorption spectrum of melanin. At each wavelength, a range of energy densities was examined. Skin biopsies taken from laser-exposed sites were examined histologically in an attempt to establish whether optimal laser parameters exist for destroying pigment cells in skin. Of the five wavelengths examined, 504 nm produced the most pigment specific injury; this specificity being maintained even at the highest energy density of 7.0 J/cm^2. Thus, for the destruction of melanin-containing cells in the epidermal compartment, 504 nm wavelength appears optimal. *J Invest Dermatol 92: 717–720, 1989*

Benign cutaneous pigmented lesions, such as lentigines, café au lait macules, and nevi, are commonly seen in dermatologic practice. Over the last two decades, there have been several reports describing the successful removal of these pigmented lesions by a variety of lasers such as the excimer (351 nm), argon (488, 514 nm), ruby (694 nm), Nd:YAG (1060 nm), and CO_2 (10,600 nm) lasers [1–5]. Pigmented lesions treated by laser have included lentigines [2], nevi [6,7], melanomas [3,4,8–10], oral hypermelanosis of Peutz-Jeghers syndrome [11], the nevus of Ota [3,12], and a lentigo maligna [13]. The pigment depth of these laser-treated lesions have also varied significantly, from superficial lentigines in the epidermis to lesions lying deep in the reticular dermis like the nevus of Ota.

Previous studies reporting "successful" removal of pigmented lesions have relied on clinical assessment rather than histology and have used widely divergent wavelengths, pulse durations, energy densities, and spotsizes. There have been no effort to define laser parameters necessary for optimal removal of pigmented lesions.

To define the necessary laser parameters, we first characterized the absorption spectrum of the chromophore melanin and examined how light emitting at wavelengths that match the absorption spectrum of melanin affect pigmented cells, a process called selective photothermolysis.

Melanin, an endogenous cutaneous pigment, which is most concentrated in the basal layer of the epidermis, has an absorption spectrum that is highest in the ultraviolet range and gradually diminishes toward the infrared [14]. Melanosomes, which are melanocyte-specific organelles, densely packed with melanin, are predominantly found within melanocytes. They vary in size according to their genetic origin; black skin typically containing larger melanosomes than lightly pigmented, white skin [15]. Based on melanosome size, the calculated thermal relaxation time for these organelles is in the 50–100-nsec range. On the other hand, melanocytes are approximately 7-μm in diameter, with thermal relaxation times around 1 μsec [16]. Thermal relaxation time in both instances is defined as the time taken for a structure to cool to 50% of its peak temperature immediately after laser exposure [16,17].

Recent studies have applied the technique of selective photothermolysis to specifically destroy melanosomes using the XeF pulsed excimer in vitro and the Q-switched ruby lasers in vivo [18,19]. Histologically, both these studies demonstrated melanosomal injury that was associated with disruption of melanocytes as well as melanin-containing basal keratinocytes. In addition, there was also evidence of follicular damage after exposure of pigmented guinea pig skin to the Q-switched ruby laser [19].

Because these studies only used single wavelengths at distal ends of the melanin absorption spectrum, the excimer at 351 nm and the ruby at 694 nm, this study was undertaken to examine wavelengths that fall within the major absorption spectrum of melanin. We compared histologic changes after exposure of pigmented pig skin to five wavelengths in our attempt to establish whether optimal laser parameters exist for destroying pigment cells in skin.

MATERIALS AND METHODS

Three miniature black pigs, each weighing approximately 80 lb, were anesthetized using a mixture of ketamine (27.2 mg/kg), atropine (0.068 mg/kg) and xylazine (2.5 mg/kg). Hair was shaved before laser irradiation.

A Candela flashlamp-pumped tunable dye laser, with a pulse duration of 300 nsec, was tuned to 504, 590, 694, 720, and 750 nm

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Reprint requests to: Oon Tian Tan, M.D., Department of Dermatology, Boston University School of Medicine, 80 East Concord Street, Boston, MA 02118.

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using a variety of dye mixtures supplied by the manufacturer. Each wavelength was measured using an Optometrics monochrometer with an accuracy of ±5%. Energy densities ranging from 0.25 to 3.0 J/cm² at 0.25 J/cm² increments, and at 4.0, 5.0, 6.0, and 7.0 J/cm² were delivered to pigmented skin at a spotsize of 3 mm diameter. The skin was irradiated 12 separate times at each energy density for each of the five wavelengths tested. The energy density was measured using a Scientech Energy Meter calibrated to an accuracy of ±10%. Two, 3-mm skin biopsies were taken at each energy density from each of the five wavelengths immediately, and at 4, 16, 23, and 33 d after laser exposure. The biopsy specimens were preserved in 10% formaldehyde, processed for light microscopy, and stained with hematoxylin and eosin.

RESULTS

Clinical Graying of the skin was noted immediately after laser exposure at all sites starting at an energy density as low as 0.5 J/cm² at 504 nm and at 0.75 J/cm² at 590, 694, 720, and 750 nm (Table I). This graying was no longer detectable 3–4 d after laser exposure when all laser-irradiated skin appeared normally pigmented.

White hairs, instead of black, were observed at some of the laser exposed sites on the third and fourth days after laser irradiation. Hair changes were observed at several different energy densities and varied according to the wavelength used to irradiate the skin (Table I). White hairs were present at sites exposed to 2.75 J/cm² at 590 nm, 3.0 J/cm² at 694, 720, and 750 nm, and 6.0 J/cm² at 504 nm (Table I).

Sixteen days after laser exposure, procelain white, completely depigmented macules, each measuring 3 mm in diameter, appeared at the irradiated sites (Table I). The energy density required to produce these white macules appeared to be controlled by the wavelength: 4.0 J/cm² at 504 and 720 nm, 2.75 J/cm² at 750 nm, 2.5 J/cm² at 590 nm, and 1.5 J/cm² at 694 nm (Table I). By 23 d, however, it appeared that depigmentation was observed at even lower energy densities than those noted at 16 d at all wavelengths but 694 nm; occurring at 2.25 J/cm² and upward at all four wavelengths (Table I).

The rate at which repigmentation occurred also varied according to the wavelength (Table I). Skin exposed to 504 nm irradiation repigmented first, being complete by 33 d, whereas 750 nm irradiated skin repigmented last, taking up to 6 wk for its pigment to return to normal. Repigmentation of skin exposed to 590, 694, and 720 nm occurred in this sequence at times intervening those of 504 nm and 750 nm.

Histology Light microscopic examination of biopsies taken immediately after laser exposure revealed the presence of focal epidermal edema at energy densities as low as 0.75 J/cm² (Table II). Vacuolization of individual cells scattered along the basal layer was observed as the energy density increased to 1.0 J/cm² for 504 nm, 1.25 J/cm² at 590 nm, 1.5 J/cm² for 694 and 720 nm, and 1.75 J/cm² at 750 nm. The most striking change observed was that melanin granules within these vacuolated cells marginated to the periphery of the cell (Fig 1).

Not only did the number of vacuoles increase with increasing energy density, but they also began to coalesce to form vesicles. This occurred at 2.5 J/cm² at 504 and 590 nm, 3.0 J/cm² at 694 nm, and 4.0 J/cm² at 720 and 750 nm. Exposure of skin to even higher energy densities at all wavelengths but 504 nm produced subepidermal clefts that was accompanied by epidermal necrosis; occurring at 5 J/cm² for 590 and 694 nm and 4.0 J/cm² for 720 and 750 nm (Fig 2, Table II). Interestingly, no subepidermal clefts or epidermal necrosis were observed after exposure of skin to 504 nm irradiation; not even at the highest energy density of 7.0 J/cm². In addition to epidermal injury, dermal damage consisting of collagen bundle separation accompanied by changes in the tinctorial quality of the bundles were also observed in biopsies taken from skin exposed to 7.0 J/cm² at four (590, 694, 720, and 750 nm) of the five wavelengths tested. The extent of dermal injury appeared dependent on the wavelength; the most severe occurring at 750 nm.

Vascular changes consisting of erythrocyte cell packed blood vessels were also observed in 504 and 590 nm irradiated skin.

Table I. Fluence (J/cm²) Required to Produce Specific Clinical Changes at the Five Wavelengths

Wavelength (nm)	Immediate Epidermal Graying (J/cm²)	White Hairs (4 d) (J/cm²)	(16 d)	White Macules (23 d) (J/cm²)	(33 d)
504	0.50	6.00	4.00	2.25	—
590	0.50	2.75	2.50	2.50	4.00
694	0.75	3.00	1.50	2.25	4.00
720	0.75	3.00	4.00	2.25	3.00
750	0.75	3.00	2.75	2.25	2.75

Table II. Correlation of Fluence with Histologic Changes Observed in Biopsies Taken Immediately After Laser Exposure

Wavelength (nm)	Edema (J/cm²)	Vacuolization (J/cm²)	Vesicle (J/cm²)	Epidermal Necrosis (J/cm²)	Subepidermal Separation (J/cm²)
504	0.75	1.00	2.50	—	—
590	0.75	1.25	2.50	2.25	5.00
694	1.00	1.50	3.00	2.75	5.00
720	1.00	1.50	4.00	2.75	4.00
750	1.25	1.75	4.00	2.75	4.00

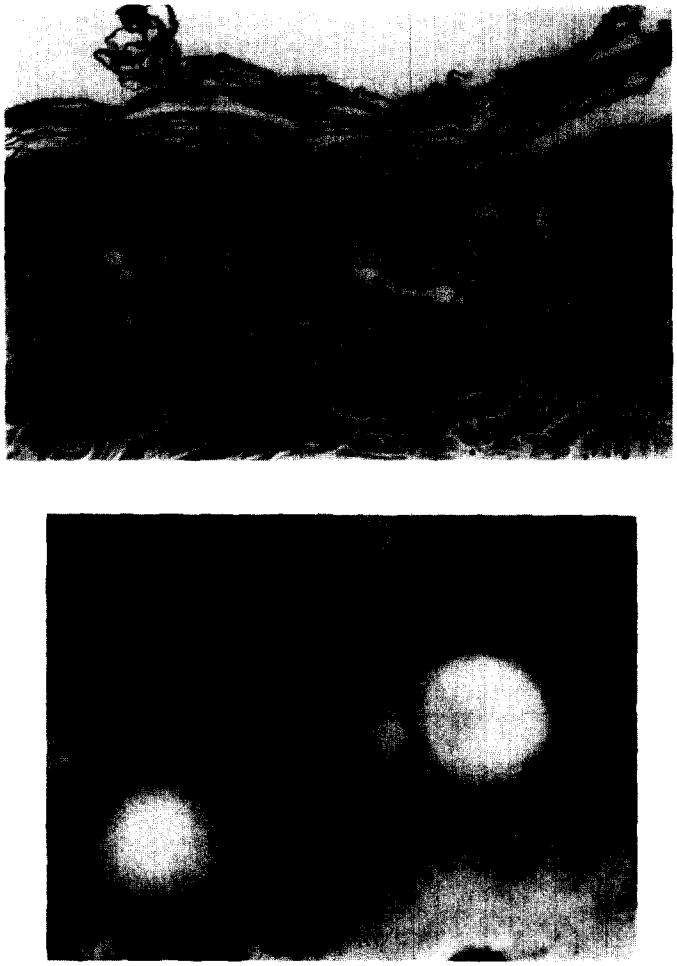

Figure 1. *A:* Vacuolization or ballooning of melanin-containing cells is observed. *B:* Note that the melanin marginates to the periphery of the vacuolated cells *(arrowheads)*. Immediately after laser irradiation of skin exposed to 7.0 J/cm² at 504 nm wavelength. (×20)

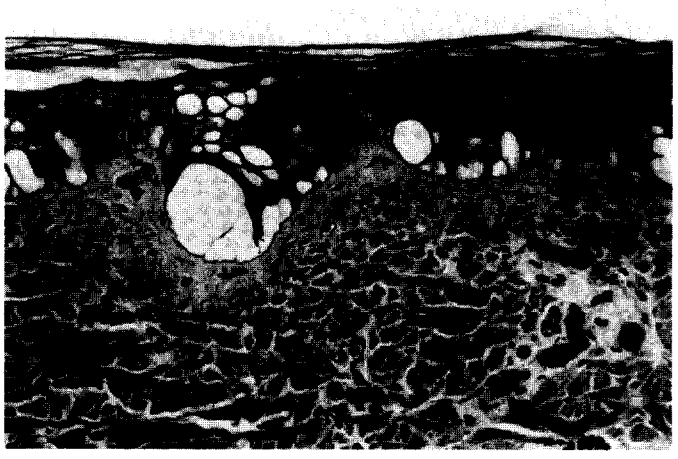

Figure 2. Vacuolated cells coalesce to form subepidermal bullae at higher energy densities of 7.0 J/cm² at 720 nm. This is also associated with full thickness coagulative necrosis of the epidermis, shown here by pyknosis of keratinocyte nuclei. (×20)

Several changes were evident in biopsies taken 4 d after laser irradiation. A regenerating hyperplastic epidermis covered by scale-crust packed with melanin granules was observed in these biopsies. The underlying basal layer was pale and the degree of melanization of these cells appeared diminished. Pigmentary incontinence also accompanied these changes and was evident in these biopsies; its severity increased with increasing energy density and wavelength.

No melanin-containing cells were observed at the center of biopsies taken on the 16th d after exposure of skin to 1.5 J/cm² at 694 nm, 2.5 J/cm² at 590 nm, 3.0 J/cm² at 750 nm, and 4.0 J/cm² at 504 and 720 nm (Fig 3). Away from the center, however, dendritic melanocytes were present at the periphery of these biopsies where they appeared to aggregate to the base of the rete ridges (Fig 4).

Histologically, melanization of the entire basal cell layer appeared complete by 33 d at all five wavelengths. The melanin in these cells was confined primarily to dendritic melanocytes. Although apparently complete, melanin concentration within these regenerated pigment cells appeared less dense than that of control nonlaser-exposed melanin-containing cells.

Figure 3. At 16 d after laser irradiation no melanin is present in epidermal cells in skin exposed to 7.0 J/cm² at 504 nm. (×40)

Figure 4. Melanization of the epidermis begins with dendritic melanocytes *(arrowhead)* migrating from the periphery toward the center of the lased specimen at 23 d after exposure to 3.0 J/cm², 750 nm. (×40)

Therefore, summarizing the results, it is evident from data presented that epidermal pigment cells can be selectively damaged by laser irradiation at wavelengths ranging from 504 to 750 nm. Several points, however, need to be highlighted. Of the five wavelengths examined, 504 nm produced the most pigment-specific injury even at the highest energy density tested of 7.0 J/cm². Although 590, 694, 720, and 750 nm irradiation also specifically damaged melanin-containing cells, coagulation necrosis of the epidermis also accompanied these changes; occurring at 2.25 J/cm² at 590 nm and 2.75 J/cm² at the three remaining wavelengths (Fig 5).

Comparing the energy density required to produce pigment specific cell injury at the different wavelengths, this also appeared to be wavelength dependent. Exactly similar histologic changes were produced at lower energy densities in skin exposed to the green wavebands (504 nm) than to the red (750 nm) (Table II). For example, vacuolization, the earliest histologic change observed, was evident in skin exposed to 1.0 J/cm² at 504 nm, but not until 1.75 J/cm² at 750 nm (Table II).

By contrast, the opposite was true of subepidermal separation where a lower energy density (4.0 J/cm²) was needed to induce this change at 720 and 750 nm than at 590 and 694 nm (5.0 J/cm²). Interestingly, no subepidermal separation was detected in skin exposed to 504 nm, not even after exposure to 7.0 J/cm².

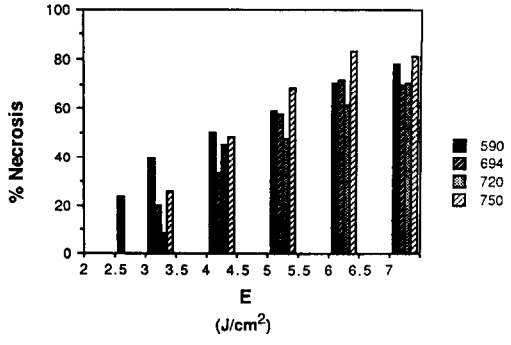

Figure 5. Correlation of degree of epidermal necrosis with fluence (J/cm²). Note that greater than 50% epidermal necrosis was observed after exposure of skin to 5 J/cm² at 590, 694, 720, and 750 nm. Such extensive injury delayed repigmentation of the epidermis by 7 d when compared with injury produced at 4.0 J/cm² and less.

The energy density producing clinical depigmentation of the irradiated skin was similar to that which resulted in histologic pigment cell injury. Light microscopic examination of these biopsies revealed an absence of melanin only at the center of the laser irradiated field. Pigment was present, however, in dendritic melanocytes at the periphery of these specimens.

In spite of a good correlation between clinical depigmentation and histologic loss of epidermal pigment, the same did not hold true for repigmentation of the laser-exposed sites. In the case of 504 nm irradiation, although progression of dendritic melanocytes toward the center was observed histologically in biopsies taken at 23 d, repigmentation was not clinically evident until 33 d after laser exposure.

DISCUSSION

Whether this experimental data on response and repair of normal pigmented skin to laser exposure can be transposed to the treatment of pigmented lesions needs to be examined further. It is clear from this study that 504 nm is most effective for destroying pigment in the epidermis compared with the other four wavelengths tested. Not only was a lower energy density needed to destroy melanocytes and melanin-containing cells but pigment cell damage also appeared more specific at this wavelength. There was less nonspecific epidermal and dermal damage induced, thus, decreasing healing time.

Lentigines and café au lait macules are hyperpigmented lesions with increased concentrations of melanin along the dermoepidermal junction in basal keratinocytes and melanocytes [20]. Because pigment concentration is "superficial," it would be reasonable to assume that 504 nm irradiation should effectively ablate it. Theoretically, it should also be possible to ablate pigment deep in the dermis, such as the nevus of Ota and Ito, using longer wavelengths. It is unclear at present which wavelength will be optimal to achieve such depths of penetration. It is known from previous work that the depth of penetration of light is dependent on its wavelength and longer wavelengths are likely to be required.

For the destruction of epidermal compartment melanin-containing cells, laser wavelength of 504 nm and energy densities of 0.5 to 6.0 J/cm^2 appear optimal. It is of interest that the skin exposed to these laser parameters repigment normally without scar formation after injury, thus diminishing the likelihood of permanent pigment loss. This will be of utmost importance when this laser is used to treat benign cutaneous pigmented lesions where the "ideal" treatment would be one where only the cells in the hyperpigmented lesions are destroyed without permanently damaging adjacent epidermal and dermal structures. Data from this study suggests that it is likely that this end result can be achieved in the treatment of superficial benign cutaneous pigmented lesion. A careful balance in the laser parameters used will have to be structured between the degree of injury imposed on cells in the hyperpigmented lesion with that induced in adjacent "normal" epidermis and dermis. Such a balance should lead to the removal of the hyperpigmented lesion without permanently destroying normal adjacent epidermal and dermal structures.

REFERENCES

1. Lane RJ, Linsker R, Wynne JJ, Torres A, Geronemous RG: Ultraviolet laser ablation skin. Arch Dermatol 121:609–617, 1985
2. Ohshiro T, Maruyama Y: The ruby and argon lasers in the treatment of naevi. Ann Acad Med Singapore 12:388–395, 1983
3. Goldman L, Igelman JM, Richfield DF: Impact of the laser on nevi and melanomas. Arch Derm 90:71–75, 1964
4. Bilik R, Kahanovich S, Rubin M, Rothem A, Geiernter I, Kaplan I: Morbidity and recurrence rates after surgical treatment of malignant melanoma by scapel versus CO_2 laser beam. Surg Gynecol Obstet 165:33–338, 1987
5. Goldman L, Nath G, Schindler G, Fidler J, Rockwell RJ: High-power Neodymium-YAG laser surgery. Acta Derm Venereol (Stockh) 53:45–49, 1973
6. Landthaler M, Haina D, Waidelich W, Braun-Falco O: Argon laser therapy of verrucous nevus. Plast Reconstr Surg 74:108–111, 1984
7. Apfelberg DB, Maser MR, Lash M: Extended clinical use of the argon laser for cutaneous lesions. Arch Dermatol 115:719–721, 1979
8. Goldman L: Surgery by laser for malignant melanoma. J Dermatol Surg Oncol 5:2 141–144, 1979
9. Wagner RI, Kozlor AP, Moskalik KG, Khachuturyan LM, Pertsov OL: Laser therapy of human benign and malignant neoplasms of the skin. Acta Radiol 14:417–423, 1975
10. Kozlov AP, Moskalik KG: Pulsed laser radiation therapy of skin tumors. Cancer 46:2172–2178, 1980
11. Ohshiro T, Maruyama Y, Nakajima H, Mima M: Treatment of pigmentation of the lips and oral mucosa in Peutz-Jeghers' syndrome using ruby and argon lasers. Br J Plast Surg 33:346–349, 1980
12. Apfelberg DB, Maser MB, Lash H, Rivers J: The argon laser for cutaneous lesions. JAMA 245:2073–2075, 1981
13. Arndt KA: Argon laser treatment of lentigo maligna. J Am Acad Dermatol 10:953–957, 1984
14. Anderson RR, Parrish JA: The optics of human skin. J Invest Dermatol 77:13–19, 1981
15. Szabo G, Gerald AB, Pathak MA: Racial differences in human pigmentation on the ultrastructural level. J Cell Biol 39:2(2):132a–133a, 1968
16. Anderson RR, Parrish JA: Selective photothermolysis: precise microsurgery by selective absorption of pulsed irradiation. Science 220:524–527, 1983
17. Hayes JR, Wolbarsht ML: Thermal model for retinal damage by pulsed lasers. Aerospace Med 39(5):474, 1968
18. Murphy GF, Shepard RS, Paul BS, Menkes A, Anderson RR, Parrish JA: Organelle-specific injury to melanin-containing cells in human skin by pulsed laser irradiation. Lab Invest 49:680–685, 1983
19. Polla LL, Margolis RJ, Dover JS, Whitaker D, Murphy GF, Jacques SL, Anderson RR: Melanosomes are a primary target of q-switched ruby laser irradiation in guinea pig skin. J Invest Dermatol 89(3):281–286, 1987
20. Lever WF, Schaumberg-Lever G: Histopathology of the Skin, 6th ed. Philadelphia, J.B. Lippincott, 1983, pp 668–697