In Vivo Spectrophotometric Evaluation of Normal, Lesional, and Laser-Treated Skin in Patients with Port-Wine Stains

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The influence of patient age and argon laser therapy on port-wine stains (PWS) was studied quantitatively in 16 patients aged 15–64 years using a spectrophotometer and computer graphics/statistics program. Normalized reflectance curves revealed a 10–20% decrease with age in the reflectance of normal skin from 400 nm to 650 nm, with an even more pronounced reflectance decrease in the region of peak deoxyhemoglobin absorption at approximately 555 nm. In each patient, PWS reflectance was less than that in the normal skin, as expected, and the average discrepancy increased with age from approximately 25% to 50%, with further reduction at 555 nm. The data suggest that with advancing age, both normal skin and PWS have a greater total hemoglobin content and an increased proportion of deoxyhemoglobin, consistent with increasing vascular dilation and tortuosity; and that the age-associated changes in PWS are an exaggeration of those in normal skin. Laser-treated PWS in both young and old patients had reflectance curves indistinguishable from those of untreated PWS in young patients. This implies, contrary to published clinical impressions, that in the absence of scarring the results of argon laser therapy are the same in young and old patients, but that only older patients experience a significant color shift in the lesion.

The nevus flammeus is a common congenital lesion manifested primarily in the papillary dermis [1]. In the newborn, it is flat, pink to salmon colored, and well circumscribed [2]. A small percentage of these capillary malformations continue past the first year and evolve slowly with age to an ectatic and dilated superficial venous plexus, resulting clinically in a darker, more purple, and raised lesion [3]. These nonregressing lesions, or port-wine stains (PWS), affect the face or neck in the majority of patients and often constitute a major psychosocial problem [4]. Hypertrophy of involved facial areas and bleeding after minor trauma are additional problems for many patients with extensive lesions; glaucoma, seizures, and mental retardation alone or in combination (Sturge-Weber syndrome) can also occur in association with PWS [5]. Therapeutic trials with cryosurgery, electrocautery, dermabrasion, tattoo with skin color pigments, skin grafts, and ionizing radiation all have had limited success and/or undesirable side effects [6]. Specifically formulated makeup is sometimes helpful, especially for macular lesions in females, but is less useful for males or for raised lesions [5].

Argon lasers, which have been used for over 15 years as an

Abbreviations:

PWS: port-wine stain

optical cautery device to treat retinal detachment [7], have also been applied in the treatment of PWS [8,9]. Theoretically, the argon laser is especially well suited to the treatment of cutaneous vascular lesions since its major output lines at 488 nm and 514 nm (blue-green visible light) are absorbed strongly by hemoglobin [10]. Lasers exert their effects on tissue when the high-intensity incident beam of photons is absorbed by a pigment (chromophore) with an appropriate absorption spectrum, releasing heat at the site of this photo-thermal interaction [11]. Hence, the preferential absorption of argon laser energy in the skin by intravascular hemoglobin focuses the resultant thermal injury on the abnormal dermal vasculature, and reduces the injury to surrounding cutaneous structures. Indeed, treatment of PWS is at present the only medical application for the laser which attempts to utilize its wavelength-dependent preferential absorption to selectively destroy one anatomic target within a complex tissue.

Early reports of argon laser treatment of PWS noted approximately 71% favorable results, a major advance over other modalities, but approximately 12% of patients unpredictably experienced hypertrophic scarring or an otherwise unwanted outcome [12]. Subsequently, it was observed in 62 patients that the patient's age, the lesional color, and certain vascular parameters measured in histologic sections may be used to predict therapeutic response [13]. Statistically, the older patient with a dark red or purple lesion and with biopsy-documented ectatic and dilated superficial vascular plexus responds to argon laser therapy with a greater shift of lesional color toward normal and with less risk of hypertrophic scarring than does the younger patient with a pink-red lesion and a minimally distorted vascular plexus. Knowledge of these relationships has enabled physicians to avoid treating high-risk patients and has led to useful modification of argon laser therapy [13,14]. However, a small proportion of PWS patients continue to respond unfavorably to the laser even when screened with a lesional biopsy.

Cutaneous spectrophotometry, a technique that measures reflected monochromatic light from skin, can be used as a noninvasive method to characterize the in vivo pigments. We have used this technique on the normal, lesional, and laser-treated skin of PWS patients to study the age-related cutaneous changes of PWS patients and to develop insight into the PWS lesion, its response to argon laser treatment, and the possibilities for alternative treatments.

MATERIALS AND METHODS

Patients

Sixteen patients with PWS treated in the Beth Israel Hospital Laser Unit were invited to participate in the study. Patients aged 15–64 years were divided into 3 age groups corresponding to perceived risk categories [13]: ≤ 20 years (4 patients, average age 16.8 years), 21–40 years (6 patients, average age 20.3 years), and ≥ 41 years (6 patients, average age 52.8 years). With one exception, the PWS involved the face or neck, with the majority on the cheek and/or forehead. Untreated lesions ranged from light pink and macular to dark purple and raised, and the laser treatment results were in all instances similar to those anticipated on clinical grounds [13].

Treatment

A Coherent Radiation Model No. 1000 argon laser was used according to described protocols [13,14] at least 3 months before spectrophotometric evaluation. Briefly, consecutive adjacent areas of the PWS were treated with a scanning motion through a hand-held fiber optic device using a 1 mm-diameter laser beam at a power setting of 1.5–2.0 W and a 0.2-s pulse duration (equivalent to approximately 150–200 W/cm^2).

Spectrophotometry Apparatus and Measurement

A Carey 17 dual-beam spectrophotometer with fiber-optic conduits to a portable measurement appliance was used. The incident beam of light was at 45° to the skin and illuminated approximately 1 cm^2 of skin surrounded by a black chamber (2 × 5 × 3 cm). Reflected light was then collected by a fiber-optic conduit oriented normal to the skin and automatically compared to an internal reference beam. The resulting values were plotted continuously for wavelengths between 400 nm (violet-blue) and 650 nm (red). A Kodak white paint standard was used to generate the normalizing standard of 100% reflectance for wavelengths between 400–650 nm. Reflectance so measured and normalized is proportional to 1.00 minus the fraction of incident beam absorbed by the test skin. The wavelength range was chosen to include the major absorption maxima of oxy- and reduced hemoglobin as well as the major output lines of the argon laser (Fig 1). Normal skin adjacent or contralateral to lesional skin was also measured to serve as a control. All sites studied were representative of the lesional or normal skin.

Data Analysis

Values for raw reflectance curves were entered for wavelengths every 5 nm between 400 nm and 650 nm using a digitizing tablet. Ten thousand data points were stored and analyzed using the PROPHET timesharing system. Patient curves were first normalized by the Kodak white (100%) standard. Because the melanin absorption coefficient is largest at 400 nm in the wavelength range used, each patient's normal skin reflectance at 400 nm was adjusted to the arbitrary value of 0.15. All curves for that patient were then vertically adjusted on the logarithmic absorbance scale to compensate for differences in melanin among patients.

These normalized, adjusted curves were analyzed using the PROPHET Statistics package and average curves generated for 3 age groups (≤ 20 years, 21–40 years, ≥ 41 years) and age-related changes noted. For subsequent statistical analyses, groups were coalesced to "young" (≤ 20 years) and "old" (≥ 20 years) and compared using the Student *t*-test.

RESULTS

Reflectance values for normal skin of PWS patients show a gradual increase from 400 nm (blue-violet) to 650 nm (red) consistent with the gradual reduction in melanin absorption capacity at longer wavelengths (Figs 1, 2A). A "double-trough" interrupts this trend at the oxyhemoglobin absorption maxima 542 nm and 577 nm [15] where reflectance is reduced. Normal skin of the younger patients is characterized by 10–20% greater reflectance throughout the visible spectrum, especially for the "red" region of 500–600 nm, than is normal skin of the older patients ($p < 0.05$). In addition, the "double-trough" region of the older group is flattened relative to the younger group at the intermediate wavelength of approximately 555 nm, suggesting greater absorption by deoxyhemoglobin [15] (Figs 1, 2A).

As expected, in all patients, reflectance of PWS skin was less than that of normal skin ($p < 0.05$). For younger patients, however, PWS reflectance curves averaged 25% lower values than normal control skin (Fig 2B), while in older patients PWS curves averaged 50% lower values (Fig 2C). Moreover, in younger patients, the reflectance differences between the normal and PWS skin were greatest between 542–577 nm, the local absorption maxima of oxyhemoglobin, and disappeared by 650 nm; while in older patients, reflectance differences persisted throughout the visible spectrum and were greatest at 650 nm. The "double-trough" region was flattened in PWS compared to normal skin in both age groups, but this change was more prominent in older patients.

Older PWS skin averaged 20–30% lower reflectance than younger PWS skin ($p < 0.05$), consistent with the clinically apparent darker purple-red color of these lesions, with further separation of the curves for wavelengths > 600 nm (Fig 2D). The flattening of the "double-trough" seen in older normal skin was even more striking for older PWS skin. Thus, the age-related changes in PWS skin appear to be an exaggeration of similar changes seen in normal skin.

In both age groups the reflectance curves for the laser-treated PWS were statistically identical ($p > 0.20$) to the reflectance curve of untreated PWS in younger patients for wavelengths 400–600 nm (Fig 2F), while the reflectance curve for the treated PWS of the older patient, with a darker initial lesion, showed a greater shift ($p < 0.05$) toward his/her normal skin reflectance curve (Fig 2G).

All reflectance curve differences attributable to the presence of the PWS or to patient age were accentuated for wavelengths 600–650 nm, as compared to wavelengths 400–600 nm, i.e., at wavelengths beyond the absorption maxima of oxyhemoglobin and beyond the output lines of the argon laser (Fig 2F–G). The one exception was the difference between the PWS and normal skin of younger patients, in whom curve speararion was maximal for wavelengths 542–577 nm.

DISCUSSION

This study amplifies earlier work [16–19] demonstrating that the spectrophotometer provides a rapid, sensitive, and noninvasive means to quantify alterations in blood or other cutaneous chromophores in both normal and lesional skin. Moreover, two new concepts emerge from our data. First, differences between a patient's PWS and his/her normal skin or between PWS in young and old patients are an exaggeration of the age-related changes seen in normal skin. These changes are a reduction in the reflectance curve over a broad wavelength range consistent in part with increased blood content of the skin; and a flattening of the "double-trough" region, consistent with a greater admixture of deoxyhemoglobin in the superficial vascular plexus. This may reflect increased stagnation of blood due to increased vascular tortuosity and dilation both in the abnormal superficial plexus of the PWS and in older chronically sun-exposed facial skin. This hypothesis is consistent with the histologic findings of Barsky et al [3] who studied PWS evolution from childhood to late adulthood, and with the common clinical observation of telangiectasia of sun-damaged skin. In this hypothesis, the PWS vessels would be even more vulnerable than normal vessels to age-associated structural alterations.

Second, reflectance curves for treated PWS in all age groups are identical between 400–600 nm, consistent with the histologic studies by Finley et al [20] which showed that the dilated, ectatic vascular PWS plexus was replaced after laser treatment by a characteristic network of small-diameter venules regardless of patient age, initial lesional color, or therapeutic result. The combination of our spectrophotometric data and earlier histologic data strongly suggests that, contrary to current clinical impressions, results of argon laser therapy are comparable in young and old patients, and that older patients appear to have a better result only because the initial PWS is darker and hence offers a greater contrast to the treated area.

Reflectance differences between PWS and normal skin in older patients are greatest for wavelengths 600–650 nm. This is consistent with the presumed greater proportion of deoxyhemoglobin in the PWS, since the absorption coefficient for deoxyhemoglobin in this region is much greater than that for oxyhemoglobin [15] and the lesser absorption by melanin in longer wavelengths increases the impact of total hemoglobin absorption on the reflectance curve beyond 600 nm [11]. Increased light scattering by altered connective tissue might also contribute to the dark purple color (decreased reflectance) of the mature PWS. In contrast, in younger patients, maximal curve separation occurs in the wavelength range 542–577 nm, suggesting that a greater oxyhemoglobin content is the major difference between normal and PWS skin in this age group.
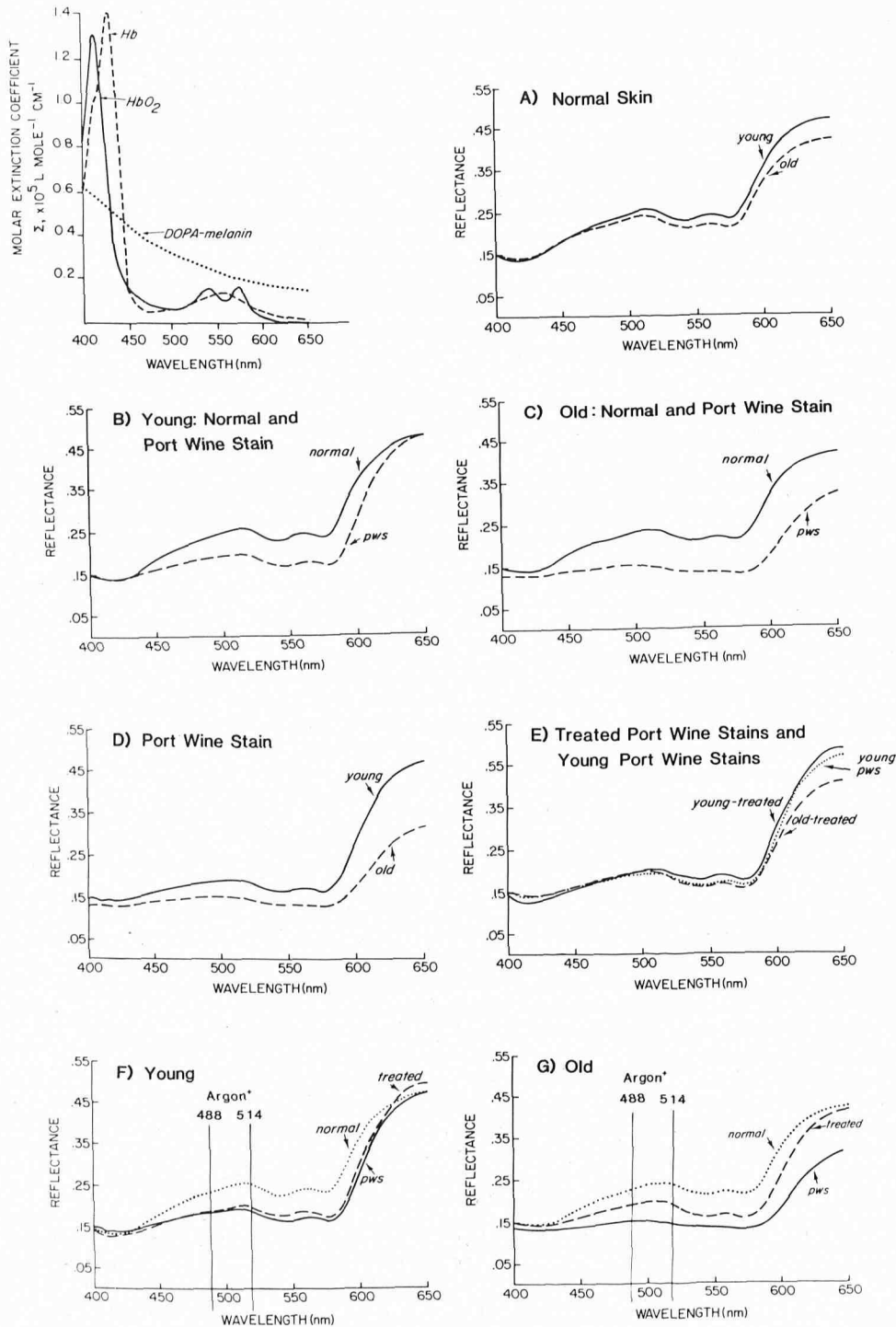
FIG 1. Absorption spectra of the major human skin pigments. Molar extinction coefficient for solutions of oxyhemoglobin (HbO_2), deoxyhemoglobin (Hb), and melanin (*DOPA-melanin*) are plotted as a function of wavelength in the visible light spectrum. The absolute height of each curve above baseline is related to concentration of the solution measured; relative absorption of the actual pigments in vivo cannot be deduced. Note that melanin absorbs throughout the spectrum, but absoprtion is less at longer wavelengths. Oxyhemoglobin has local absorption maxima at 542 nm and 577 nm; and deoxyhemoglobin at 555 nm. Beyond these local maxima, oxyhemoglobin falls rapidly to less than 0.5% of its local maxima, while deoxyhemoglobin absorption falls more gradually to approximately 5.0% of its local maxima [15]. These absorption spectra are mirrored in the in vivo cutaneous reflectance curves (Fig 2). (Modified and reproduced with permission from Anderson RR, Parrish JA: Optical properties of human skin, Photomedicine. Edited by JD Regan, JA Parrish. New York, Plenum Press, 1982, pp 147–194.)

FIG 2. Normalized mean reflectance curves of normal, lesional, and laser-treated lesional skin. *A*, Normal skin of young and old patients. *B*, Normal skin vs PWS of young patients. *C*, Normal skin vs PWS of old patients. *D*, PWS of young vs old patients. *E*, Laser-treated PWS in young and old patients vs untreated PWS in young patients. *F*, Normal, PWS, and laser-treated PWS in young patients. *G*, Normal, PWS, and laser-treated PWS in old patients. *Vertical bars* at 488 nm and 514 nm indicate the major output lines for the argon laser.

The present study does not investigate the spectrophotometer's potential usefulness in the management of individual patients, although the desirability of a noninvasive, sensitive, and specific predictor of a favorable PWS response to argon laser therapy is clear. Until the spectrophotometer or other device can be demonstrated to reliably predict treatment response, physicians must continue to advise prospective patients based on available clinical and histologic criteria [13].

Recently, development of new technology and application of advanced mathematical modeling to the optical properties of skin have further improved our ability to quantify cutaneous pigments in vivo using noninvasive methods [21–24]. Use of these sophisticated techniques may allow further insight into the character of the PWS and may aid in the development of future treatment strategies.

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Cell Cycle Kinetics of Cultured Human Epidermal Keratinocytes

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When stratified epithelia maintained in culture are used for autoradiographic studies of labeling index, the emulsion is usually placed over the uppermost strata of the culture. In many cases the distance from the basal cell nucleus to the emulsion exceeds the average pathlength of β-particle emissions from ^{14}C or ^{3}H. We describe a technique for inverting the cultures so that the emulsion can be brought into close association with the basal cells.

Attempts to label cultured human epidermal keratinocytes using a pulse of $[^{3}H]$- or $[^{14}C]$-thymidine produced labeling only at the periphery of the colonies. This was noted when emulsion was laid on top of the colonies but also when the emulsion was in close contact with the "basal cells" adhering to the plastic culture vessel. Continuous labeling of the cultures produced nearly 100% labeling of all the basal layer, i.e., central and peripheral, indicating that the central cells were also in rapid cell cycle. The results are interpreted as indicating the presence of an efficient barrier to free diffusion over the center of the colonies, presumably due to the presence of several layers of corneocytes. Percent labeled mitoses (PLM) studies produced an unusual PLM curve with a

Abbreviations:
DCS: donor calf serum
DMEM: Dulbecco's modified Eagle's medium
EGF: epidermal growth factor
HPMA: 2-hydroxylpropyl methacrylate
LI: labeling index
MI: mitotic index
PLM: percent labeled mitosis
SV40: Simian Virus 40
T_c: cell cycle duration
T_m: mitotic duration
Thd: thymidine
TK: thymidine kinase
VCR: vincristine sulfate