Effects of Cryogen Spray Cooling and High Radiant Exposures on Selective Vascular Injury During Laser Irradiation of Human Skin

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Background: Increasing radiant exposure offers a means to increase treatment efficacy during laser-mediated treatment of vascular lesions, such as port-wine stains; however, excessive radiant exposure decreases selective vascular injury due to increased heat generation within the epidermis and collateral damage to perivascular collagen.

Objective: To determine if cryogen spray cooling could be used to maintain selective vascular injury (ie, prevent epidermal and perivascular collagen damage) when using high radiant exposures (16-30 J/cm²).

Design: Observational study.

Setting: Academic hospital and research laboratory.

Patients: Twenty women with normal abdominal skin (skin phototypes I-VI).

Interventions: Skin was irradiated with a pulsed dye laser (wavelength=585 nm; pulse duration=1.5 milliseconds; 5-mm-diameter spot) using various radiant exposures (8-30 J/cm²) without and with cryogen spray cooling (50- to 300-millisecond cryogen spurts).

Main Outcome Measure: Hematoxylin-eosin-stained histologic sections from each irradiated site were examined for the degree of epidermal damage, maximum depth of red blood cell coagulation, and percentage of vessels containing perivascular collagen coagulation.

Results: Long cryogen spurt durations (>200 milliseconds) protected the epidermis in light-skinned individuals (skin phototypes I-IV) at the highest radiant exposure (30 J/cm²); however, epidermal protection could not be achieved in dark-skinned individuals (skin phototypes V-VI) even at the lowest radiant exposure (8 J/cm²). The red blood cell coagulation depth increased with increasing radiant exposure (to >2.5 mm for skin phototypes I-IV and to approximately 1.2 mm for skin phototypes V-VI). In addition, long cryogen spurt durations (>200 milliseconds) prevented perivascular collagen coagulation in all skin types.

Conclusions: Cryogen spurt durations much longer than those currently used in therapy (>200 milliseconds) may be clinically useful for protecting the epidermis and perivascular tissues when using high radiant exposures during cutaneous laser therapies. Additional studies are necessary to prove clinical safety of these protocols.

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LASER-MEDIATED treatments of cutaneous hypervascular malformations, such as port-wine stain (PWS) birthmarks, are based on the principle of selective photothermolysis. Ideally, during this process, laser light is used to selectively heat and subsequently destroy the vascular lesion. By proper selection of pulse duration (τ₀) and wavelength (λ), thermal energy remains confined to the targeted vasculature. These therapies have proven effective, however, multiple treatments are often required, and complete clearing of the lesion is not always achieved, especially in patients with large-diameter vessels and extensive vasculature that extends deep within the dermis. Incomplete clearing of the lesion may be due to insufficient heat generation within the targeted vasculature, which is affected by pulse duration (τ₀), wavelength (λ), and radiant exposure (D₀).

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Increasing the radiant exposure potentially provides a method for a more successful treatment of PWSs. First, increased radiant exposures will raise the fluence level deep within the dermis, enabling a deeper depth of vascular
Participants were grouped by their skin phototypes (I-VI) and divided into 3 groups. Group 1 consisted of light-skinned individuals who had little to no epidermal pigmentation (phototypes I-II [n=6]). Group 2 individuals had moderately pigmented skin (phototypes III-IV [n=9]). Group 3 consisted of individuals with dark brown to black skin (phototypes V-VI [n=3]).

LASER AND CRYOGEN DELIVERY SYSTEM

Each participant was irradiated with a pulsed dye laser (Sclero-Plus; Candela Corp, Wayland, Mass) (λ=585 nm; τ=1.5 milliseconds; D0=8–30 J/cm2 at a 3-mm spot size). The cryodelivery system consisted of a fuel injector nozzle (1.3-mm orifice diameter) connected to a cryogen tank under approximately 6 atm of pressure at room temperature. The cryogen used to cool the skin was R134a (1,1,1,2-tetrafluoroethane), an environmentally compatible, nontoxic, Freon substitute (DuPont, Wilmington, Del) approved by the Food and Drug Administration with a boiling point of approximately −26°C at 1 atm. Cryogen was sprayed onto the skin surface immediately before the onset of the laser pulse in spurt durations (τspurt) that ranged from 50 to 300 milliseconds.

STUDY DESIGN

Each participant was irradiated at 4 radiant exposures (D0=8, 16, 23, and 30 J/cm2), each preceded by 3 different cryogen spurt durations (τspurt=0, 50, 100, 200, and 300 milliseconds with a 10-millisecond delay between the end of the cryogen spurt and the onset of the laser pulse). In addition, 2 sites were exposed to cryogen spurts of 300 milliseconds without laser irradiation, and 1 site was left for control (ie, not irradiated or cooled). Gross observations were made in the laboratory after tissue excision and before biopsy. Evidence of purpura, blanching, and blistering was recorded. Six-millimeter punch biopsy specimens were taken from each experimental site approximately 1 hour after the procedure. Tissue samples were fixed in 10% buffered formalin, processed for standard sectioning, and stained with hematoxylin-eosin for subsequent histologic analysis.

Each hematoxylin-eosin–stained histologic section was examined for epidermal damage, red blood cell (RBC) coagulation depth, and perivascular collagen coagulation. Epidermal damage and perivascular collagen coagulation were examined via light microscopy. We scored epidermal damage on a scale of 1 to 6, covering the range from none to greatest amount of damage: 1 indicates no change; 2, basal cell elongation; 3, cytoplasmic vacuolization and nuclei shrinkage; 4, multiple basal lacunae formation; 5, partial basal layer separation; and 6, complete epidermal ablation. To quantify the amount of perivascular collagen coagulation, we measured the percentage of vessels that contained darker staining and smoothing of surrounding collagen. Vessels counted as containing perivascular collagen coagulation contained signs of thermal injury beyond approximately 20 µm of the vessel wall. The percentage of vessels that contained perivascular collagen coagulation was computed by dividing the number of vessels that contained perivascular collagen coagulation by the total number of vessels. The number of vessels was determined by visual inspection of the histologic sections under light microscopy. Only vessels within the top 1-mm area of the skin section were included in the calculation, since no perivascular collagen coagulation was observed deeper than 1 mm. The RBC coagulation depth was measured using a micrometer reticle placed in the eyepiece of the light microscope. Accordingly, we recorded the distance between the dermoepidermal junction and the deepest vessel containing coagulated RBCs within the lumen.
RESULTS

GROSS OBSERVATIONS

Purpura, blanching, and blistering were observed in group 1 and 2 individuals. Light to moderately dark purpura formed at the lowest radiant exposure (8 J/cm²). As radiant exposure increased to 16 J/cm², the purpura became a well-defined circle corresponding to the irradiated spot size (5-mm diameter). Further increase in the radiant exposure (23–30 J/cm²) resulted in blanching within the center of the irradiated site, surrounded by purpura not extending beyond the bounds of the 5-mm spot. Blister formation was observed as wrinkling of the skin within the site irradiated at the highest radiant exposure (30 J/cm²) in some individuals. The blister formation process may have been incomplete since only 1 hour existed between irradiation and tissue excision.

The CSC prevented purpura formation only in sites irradiated with the lowest radiant exposure (8 J/cm²). In those individuals where only light purpura was observed without CSC, application of a spurt duration greater than 100 milliseconds eliminated the gross evidence of purpura.

Purpura and blanching in group 3 individuals were difficult to observe due to the darkness of the skin. Wrinkling of the skin over the irradiated site and complete epidermal ablation were observed at radiant exposures of 8 and 16 J/cm². A popping sound could be heard at the onset of the laser pulse, typical of the "popcorn effect,"¹⁹ signifying rapid vaporization of water, most likely within the basal keratinocytes.

EPIDERMAL DAMAGE

Epidermal damage was observed as epidermal basal cell spindling and elongation, cytoplasmic vacuolization, epidermal separation, and epidermal ablation. It was our goal to identify variables that did not result in irreversible epidermal damage. An epidermal damage score of 2 corresponded to very little change observed in histologic sections. Typically, only small areas of cells exhibited slight nuclei elongation. An epidermal damage score of 3 corresponded to the beginning of vaporization bubble formation within the cytoplasm. It is possible that this type of localized heating around the melanosomes could lead to dyspigmentation. Scores of 4 and higher were associated with cell membrane disruption, which would most likely lead to cell death. Therefore, we defined the epidermal damage threshold to be a score of 3 (ie, one that would avoid any changes in pigmentation or cell death).

Figure 1 shows the average epidermal score for each group. Statistical tests were computed using a 1-way analysis of variance and the Bonferroni multi-comparisons method, which corrects for experimental errors.²⁰

Since the spurt durations used in this study may be long enough to induce tissue freezing, we investigated evidence of epidermal damage in those sites cooled with 300-millisecond spurt durations and no radiant exposure. These sites showed evidence of epidermal basal cell elongation in approximately 50% of the individuals in-
dependent of skin type, resulting in an epidermal damage score of 2 for those treatment sites. Although cooling of the darkest-skinned individuals did not protect the epidermis for the radiant exposures tested, individuals with lighter skin (groups 1 and 2) sustained significant epidermal protection with CSC even at the highest radiant exposure tested.

Figure 2 illustrates epidermal protection provided by CSC at the highest radiant exposure (30 J/cm²) in a group 1 individual. Signs of epidermal damage include basal cell elongation (boxed area), cytoplasmic vacuoles (long arrows), and partial epidermal separation (short arrows). The epidermis remains completely intact.

RBC COAGULATION DEPTH

Coagulation of RBCs was observed in histologic sections as agglutination and a smoothing of RBCs within the vessel lumen. The depth of RBC coagulation increased with radiant exposure. Figure 3 shows the maximum depth of RBC coagulation vs radiant exposure without CSC for each group. Maximum coagulation depth of targeted RBCs increased logarithmically with radiant exposure. This would follow the assumption that light is attenuated approximately exponentially within the tissue. The logarithmic fit and Pearson correlation coefficient are shown in Figure 3.

Skin type played an important role in the maximum depth of RBC coagulation. Maximum RBC coagulation depth was increased to the base of the dermis in group 1 and 2 individuals; however, RBC coagulation depth could not be increased to the maximum depth of the dermis in group 3 individuals. Curves for groups 1 and 2 overlap at radiant exposures higher than approximately 23 J/cm², since at this radiant exposure the RBC coagulation depth was increased to the maximum thickness of the dermis. Measuring longer RBC coagulation depths was not possible, since subcutaneous fat tissue was not present in all histologic sections.

The CSC did not affect the maximum depth of RBC coagulation for any radiant exposure except for the lowest value (8 J/cm²). At this radiant exposure, long cryogen spurts (200-300 milliseconds) decreased the RBC coagulation depth. At all other radiant exposures (> 8 J/cm²), CSC did not affect the maximum depth of RBC coagulation. In addition, the signs of RBC coagulation within the capillaries immediately below the dermoepidermal junction did not decrease even at the longest spurt duration administered (300 milliseconds) when used with radiant exposures greater than 8 J/cm².

PERIVASCULAR COLLAGEN COAGULATION

Perivascular collagen coagulation appeared as darker staining and smoothing of collagen induced by heat transfer originating from vessels primarily located within the papillary dermis. As radiant exposures were increased, greater numbers of vessels showed signs of perivascular damage, and collagen coagulation became interconnected among multiple vessels and diffuse in nature. Diffuse areas of perivascular collagen coagulation...
induced by heat transfer away from the vasculature. B, There are no signs of perivascular collagen coagulation.

Figure 4 illustrates an example where no collagen coagulation is observed in an individual irradiated at the highest radiant exposure (30 J/cm²) in conjunction with CSC (300-millisecond spurt).

We sought to identify variable sets that statistically resulted in greater than 0% of vessels containing perivascular collagen coagulation. The asterisks in Figure 5 indicate those values that are statistically greater than 0 (α = .05) using a 1-way analysis of variance and Bonferroni multicomparsion method to correct for experimental errors.20 Significant levels of perivascular collagen coagulation occurred at radiant exposures greater than 8 J/cm² without CSC; however, CSC reduced levels of perivascular collagen coagulation. Prevention of perivascular collagen coagulation at the highest radiant exposures (30 J/cm²) occurred using 200- to 300-millisecond spurt durations for groups 1 and 2, whereas only 100-millisecond spurt durations were necessary for group 3. In addition, the amount of collagen coagulation was less in group 3 than in groups 1 and 2.

**COMMENT**

The levels of epidermal damage, RBC coagulation depth, and perivascular collagen coagulation were characterized for light-skinned (groups 1 and 2) and dark-skinned individuals (group 3). Epidermal damage scores for group 2 were only slightly higher than that for group 1, whereas those for group 3 were much higher. The RBC coagulation depth and perivascular collagen coagulation for groups 1 and 2 were similar, with a slight increase in the perivascular damage for group 2 individuals compared with group 1. Epidermal melanin content directly affects all 3 damage types. Increased epidermal melanin content results in increased risk for epidermal injury and a decrease in dermal fluence levels, leading to decreased RBC coagulation depth and decreased perivascular tissue damage. However, the individuals in this study were grouped based on their skin prototype, which is not a direct measure of the individual's melanin content. In fact, individuals of skin types I through IV can have similar melanin concentrations,18 whereas individuals of skin types V through VI always have dark brown to black skin (ie, high melanin concentrations). Therefore, we discuss the results of this study for light-skinned individuals (groups 1 and 2) and dark-skinned individuals (group 3) separately.

**LIGHT-SKINNED INDIVIDUALS (GROUPS 1 AND 2)**

An important finding of this study was that for light-to-medium-pigmented skin (groups 1 and 2), depth of RBC coagulation could be significantly increased using high radiant exposures in conjunction with CSC while maintaining selective vascular injury represented as the prevention of thermal injury to the epidermis and perivascular collagen. Thermal damage to these tissue structures previously placed a radiant exposure threshold on cutaneous vascular therapy, thus limiting the maximum depth of vascular injury. Increased depths of vascular injury may prove beneficial in treating previously unresponsive patients and decreasing the number of treatments needed to achieve clearing of the lesion.

A number of theoretical and clinical studies have investigated the maximum depth of selective vascular injury for pulsed dye lasers at 585 nm in normal skin. Using a theoretical model, Verkruysse et al10 concluded that the maximum depth for vascular injury was 1.05 mm. This was the depth at which the heat generation rate within the blood was equal to that within the epidermis. Therefore, this model did not take into account thermal diffusion and influences of initial skin temperature distribution. Previous clinical studies have also determined the maximum depth of vascular injury in normal human skin at 585 nm. Koster et al13 reported an increase in depth of vascular injury due to increased radiant exposures in normal breast and abdominal skin of white women. The depth of vascular injury was increased from approximately 0.98 mm at 5 J/cm² to 2.56 mm at 10 J/cm². However, at the maximum radiant exposures of 9 and 10 J/cm², vacuolization and necrosis were observed in the epidermis as well as "damage to adnexal structures and coagulative changes within dermal colla-
One animal model determined a depth of vascular injury of 1.8 mm at 10 J/cm² in albino mini pigs. To our knowledge, this is the first in vivo study to investigate the effects of high radiant exposures (>10 J/cm²) in conjunction with CSC. This study reports an increase in the RBC coagulation depth from approximately 1.0 to 1.5 mm at 8 J/cm² to the maximum depth of the dermis at approximately 2.5 mm at radiant exposures of 23 to 30 J/cm². The depth of RBC coagulation agrees well with similar studies that found a maximum vascular damage depth of 1.8 mm at 8 J/cm². The maximum RBC coagulation depth measured in this study may have been much larger if the dermis were thicker. At the maximum radiant exposure levels, vascular damage was observed within the subcutaneous fat.

Although we reached the maximum depth of damage within the dermis in normal skin at radiant exposures of only approximately 20 J/cm², there may be a need for higher radiant exposures to treat vascular lesions, such as PWSs. It has been shown that vascular damage depth is lower in PWS skin than in normal skin due to the increased blood vessel concentration within the dermis. Hohenleutner et al found a mean ± SD coagulation depth of 0.37 ± 0.17 mm in patients with PWS skin when irradiated at 585 nm using 6- to 8-J/cm² radiant exposures. Theoretical models also predict the maximum depth of vascular injury in PWS skin at 585 nm to be less than in normal skin, with reported values of 0.45, 10, 21, 22

This study also reports the elimination of nonspecific thermal damage (ie, epidermal necrosis and perivascular collagen coagulation), which was previously reported by other investigators to occur with increasing radiant exposures. Perivascular collagen coagulation observed at high radiant exposures without CSC was eliminated when using spurt durations of 200 to 300 milliseconds. In addition, long spurt durations protected the epidermis from thermal injury for radiant exposures even as high as 30 J/cm². This is in agreement with a similar ex vivo study on normal human skin, where long spurt durations protected the epidermis from radiant exposures as high as 30 J/cm².

The mechanism for long cryogen spurts (>200 milliseconds) preventing perivascular tissue damage is most likely due to induced cooling of the dermal tissue. Longer cryogen spurts eliminated perivascular tissue damage, without a decrease in the depth of RBC coagulation for increased spurt durations at all radiant exposures except 8 J/cm². At this radiant exposure, longer cooling times reduced purpura and RBC coagulation depth, which, without CSC, was limited to the more superficial blood vessels located within the top 1.3 mm of the skin (Figure 3). It is reasonable to assume that the temperature within the blood vessels at this radiant exposure (8 J/cm²) was just above the damage threshold (approximately 75°C); however, at the next highest radiant exposure (16 J/cm²; a 2-fold increase), a much higher temperature would be generated within the blood (>100°C). Cryogen-induced cooling for the maximum spurt durations used in this study would decrease the temperature of the dermal tissue to no less than approximately 10°C (approximately 25°C decrease) near the surface at 150 µm. Any cooling of the blood vessels at the lowest radiant expo-
sure would result in decreased purpura formation and the elimination of RBC coagulation. However, cooling the superficial blood vessels at any higher radiant exposure (≥16 J/cm²) would not decrease the blood temperature below the damage threshold.

The cryogen film does not substantially attenuate the incident laser light and, therefore, is not a likely factor for the reduction of perivascular injury. The residual cryogen film transmits approximately 90% of the light. Frost formation has been shown to form on the skin surface approximately 100 milliseconds after the end of the cryogen spurt. The laser pulse in this study was delivered 10 milliseconds after the end of the laser pulse, and therefore, frost formation did not affect the fluence levels within the tissue.

These findings suggest that some cooling of the dermis can be beneficial. This is in contrast with the previous belief that required spurt durations be sufficiently short to avoid cooling of dermal blood vessels. Controlled spurt durations (ie, controlled precooling times) allow control over the initial skin temperature profile so that superficial tissues are cooler than deeper dermal tissues. Adjustment of the spurt duration determines the depth of cooling. Partially cooling the superficial dermal vessels prevents perivascular collagen coagulation caused by excessive fluence levels observed at these shallow depths. However, deeper dermal vessels remain warmer, allowing injury to the targeted vasculature. Alteration of the initial skin temperature profile offsets the exponential heat generation rate profile of the laser irradiation, thus evenly distributing the temperature increase within the vascular lesion over all depths.

A recent theoretical study has also concluded that some cooling of the dermis may be beneficial. The authors use a slightly different argument that cooling selectivity relies on the temperature difference between the targeted dermal blood vessels and epidermal melanin. They showed that the maximum temperature difference between dermal blood vessels and epidermal melanin occurs for spurt durations from 170 to 300 milliseconds, depending on the depth of the lesion.

Thus far, we have assumed that one should avoid perivascular collagen coagulation; however, there may be instances where this effect is beneficial. Wrinkle reduction and treatment of hypertrophic scars rely on thermal injury to surrounding collagen. Therefore, heat diffusing out of the blood to the surrounding collagen may produce effects beneficial to other applications.

Although long cryogen spurts preserve selective vascular injury when using high radiant exposures, they seem to induce some epidermal injury in the absence of laser irradiation. At the shorter cooling times currently used in the clinical setting, no tissue freezing is expected to occur; however, it is possible that as cooling times are increased, some freezing of tissue could occur. The reason for the tissue freezing may be due to the cryogen pool that resides on the skin surface following the spurt termination. It has been shown that the cryogen spray forms a pool that resides on the surface several times longer than the spurt duration itself. Torres et al showed that airflow could be used to control the cryogen pool residence time by increasing its evaporation rate. Therefore, it may be possible to avoid tissue damage due to long cryogen spurts by eliminating the cryogen pool residing on the skin surface using such a technique. Further studies are needed to investigate the elimination of tissue damage by controlled cryogen exposure times.

DARK-SKINNED INDIVIDUALS (GROUP 3)

Selective vascular damage could not be maintained while using increased radiant exposures in this group of individuals since the epidermis could not be protected. Even at the lowest radiant exposure used in the study (8 J/cm²), considerable thermal damage to the epidermis occurred at the longest cryogen spurt duration (300 milliseconds). The RBC coagulation depth was increased by using higher radiant exposures; however, it was not increased to the same extent as in the individuals with lighter skin. This was due to lower dermal fluence levels in group 3 brought on by the higher absorption of melanin within the epidermis. Although the damage to blood vessels was seen at depths up to approximately 1.3 mm in dark skin, this coagulation depth would be much less in PWS skin due to the increased amount of dermal blood content. The depth of cleared vessels may be less than that necessary to produce a satisfactory therapeutic effect, which requires vessels photoagulated to a depth of approximately 0.8 to 0.9 mm.

This study reiterates the significant challenge in treating dark-skinned patients. Although high radiant exposures are needed to increase dermal fluence levels in dark-skinned patients, the risk of epidermal damage also increases. Either cooling of superficial skin layers needs to be increased or fluence levels within the epidermis need to be decreased. Recent studies have focused on increasing the heat transfer rates and the total heat removal during CSC by studying nozzle design and cryogen delivery methods. It has also been illustrated that shorter laser pulse durations increase the risk of epidermal damage, since heat does not have time to diffuse away from the melanin within the epidermis. It is likely that longer pulse durations used in conjunction with CSC may provide a means to decrease heat generation within the epidermis. In addition, melanin absorption decreases with increasing wavelength. Therefore, longer wavelength lasers could be used to decrease melanin absorption; however, blood absorption is also much less at these wavelengths.

STUDY LIMITATIONS

Although this study shows the feasibility of improving laser therapy of vascular lesions using high radiant exposures in conjunction with aggressive cooling, it does not prove the safety of this approach. Thermal tissue damage in this study was assessed via histologic observations. Although this is a useful technique to assess the relative amount of tissue damage among different treatment strategies, it is not an absolute measure of the long-term clinical effect. To assess the safety of using higher fluences in combination with long cooling times, long-term clinical studies are necessary. In addition, the definitions used in this study to describe tissue protection...
were based on histologic markers. These end points do not necessarily correspond to a clinical effect.

This study also points out the potential risk of using higher radiant exposures without some surface cooling. Increasing the radiant exposure by 2- to 3-fold (from 8 to 16 or 23 J/cm²) places even light-skinned patients at serious risk of epidermal injury and damage to dermal tissues. This implies that if the cooling device malfunctions and cryogen is not delivered correctly, significant injury to the patient could occur.

Lastly, there are differences between normal and hypervascular skin. First, the lower blood content of the normal skin may result in increased optical back scattering and, thus, higher heat generation within the epidermis for nonhypervascular skin. Second, as pointed out earlier in this article, penetration of light is much less in normal skin than that of hypervascular skin. Therefore, vascular injury would be confined to more superficial depths in hypervascular skin than in the normal skin studied herein.

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