Time-sequence histologic imaging of laser-treated cherry angiomas with in vivo confocal microscopy

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Objective: To chronicle the pathophysiologic changes that occur subsequent to laser treatment of vascular lesions, we used a confocal scanning laser microscope that yields high-resolution microscopic images of skin in vivo.

Methods: Cherry angiomas were treated with the 585-nm flashlamp-pumped pulsed-dye laser (PDL) and the 568-nm continuous-wave krypton laser. Repeated confocal reflectance imaging was performed before and immediately after treatment, as well as after several hours, 1 day, 2 days, 1 week, 2 weeks, 3 weeks, and 4 weeks.

Results: Before treatment, confocal images revealed dilated blood vessels ranging from 10 to 50 μm in caliber, closely spaced at 5 to 50 μm apart. After PDL treatment, amorphous cords of refractile material conformed to the shape of the original vessels, followed by dark nonrefractile spaces where the vessels once were. Inflammation and necrosis ensued, with eventual replacement after 3 weeks by normal-appearing skin. After krypton laser treatment, dark nonrefractile spaces appeared immediately, with subsequent inflammation, necrosis, and eventual healing by 4 weeks.

Conclusion: Confocal laser microscopic imaging elucidates the dynamic pathophysiologic events that occur after laser treatment of vascular lesions and has added insight into the different mechanisms of vessel damage induced by the PDL and krypton laser. (J Am Acad Dermatol 2000;43:37-41.)

The pathophysiologic changes that occur after laser treatment of vascular lesions have been assayed previously through histologic analysis of biopsy specimens taken from the treated lesions. However, because biopsy permanently changes the original lesion and eventuates in scarring, it cannot be undertaken frequently or repeatedly. Recent advances in confocal reflectance microscopy (CM) have allowed the development of a real-time confocal scanning laser microscope that yields high-resolution microscopic sectional images of skin in vivo.[1,2]

CM operates by tightly focusing a laser beam on a specific point in tissue, detecting only the light reflected from the focal point through a pinhole-sized spatial filter.[3] The beam is then scanned horizontally over a two-dimensional grid to yield a horizontal histologic sectional image. The tight focus of the laser beam and the small size of the pinhole filter,

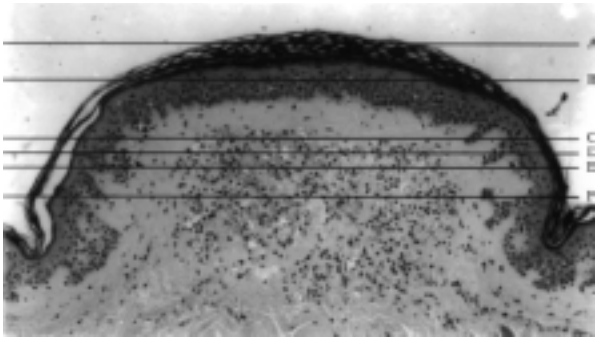

Fig 1. Cherry angioma histopathology. Depth of confocal microscopic sections from Fig 2 are drawn onto this histopathologic vertical section. (Courtesy of Thomas Flotte, MD, Dermatopathology, Massachusetts General Hospital, Boston.)

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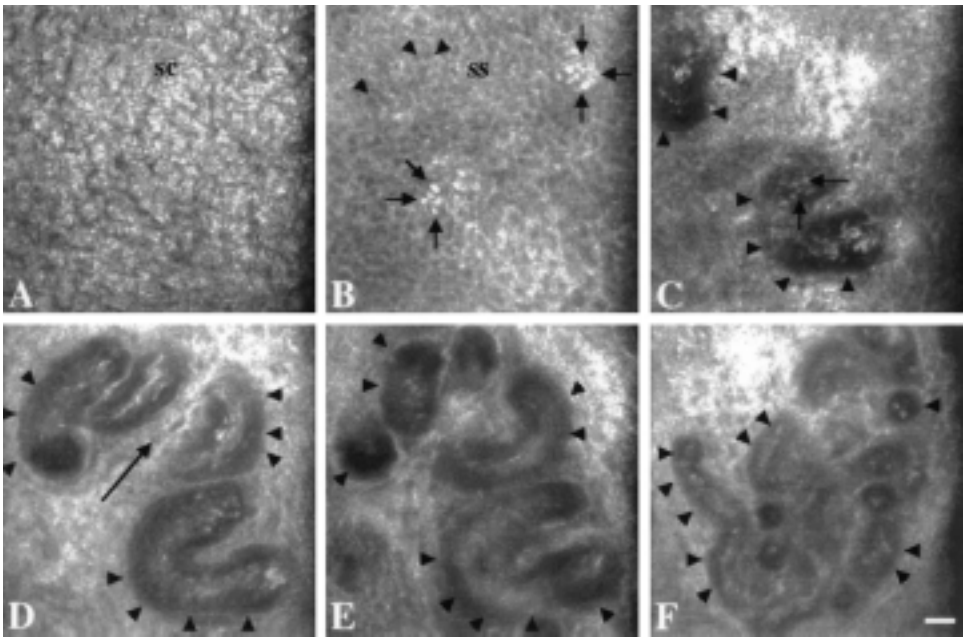

Fig 2. Cherry angioma before treatment has normal epidermis, including **(A)** stratum corneum *(sc)*, **(B)** stratum spinosum *(ss)*, and stratum basale. Basal cells (**B,** *arrows*) are brighter than surrounding spinous keratinocytes (**B,** *arrowheads*) because of reflectivity of melanin. Dilated blood vessels (**C-E,** *arrowheads*) are seen in papillary dermis between collagen bundles (**D,** *arrow*). Individual erythrocytes (**C,** *arrows*) can be visualized within vessels. Deeper in dermis, smaller caliber but more tortuous dilated vessels (**F,** *arrowheads*) are seen

respectively, determine the high lateral resolution of 0.5 to 1 μm and the axial section thickness of 2 to 5 μm. Thus images obtained by means of CM can have histologic quality with cellular and subcellular detail. Because of the limited penetration of near-infrared light in skin, the maximum depth of these horizontal sections is 300 to 400 μm, permitting imaging of the entire epidermis, papillary dermis, and uppermost reticular dermis. Images are obtained at a video rate of 15 to 30 Hz, allowing high temporal resolution in dynamic processes, such as blood cells flowing through a vessel. Infrared reflectance CM does not require staining, nor does it induce photochemical reactions. Therefore one sees living skin in its native state, without alteration, and as many times as required.[1] In this article, we report dynamic events that occur after use of pulsed-dye laser (PDL) and krypton laser treatment of microvessels.

METHODS

A subject with multiple cherry angiomas was enrolled in this study after informed consent. Several of his lesions were imaged by means of CM. One such lesion, measuring 1 mm, was treated with the flashlamp-pumped PDL (Candela, Wayland, Mass) at 585 nm, with one pulse of 5 J/cm^2 using a 5-mm spot size. A second lesion, also measuring 1 mm, was treated with the continuous-wave krypton laser (HGM Medical Laser Systems, Salt Lake City, Utah) at 568 nm with a fluence of 0.75 W, a 1-mm spot size, and an exposure duration of 1 second. Repeated high-resolution confocal imaging of the 2 treated lesions was performed immediately after treatment and after several hours, 1 day, 2 days, 1 week, 2 weeks, 3 weeks, and 4 weeks. All images were obtained by means of a commercially available near-infrared confocal reflectance microscope (Vivascope 1000, Lucid, Henrietta, NY), with a 30× water-immersion objective lens, 0.9 numerical aperture, and an 830-nm diode laser illuminating the skin with a power lower than 30 mW.

RESULTS

Cherry angioma before treatment (Figs 1 and 2)

Fig 1 shows the features of a cherry angioma on conventional histology. Underneath a normal epidermis (Fig 2, *A, B*) CM images showed dilated blood vessels ranging from 10 to 50 μm in diameter. These are tortuous and closely spaced at 5 to 50 μm apart (Fig 2, *C, D, E*). The vessels tended to decrease in caliber with increasing depth (Fig 2, *F*). Blood cells coursed rapidly through these vessels, and even in

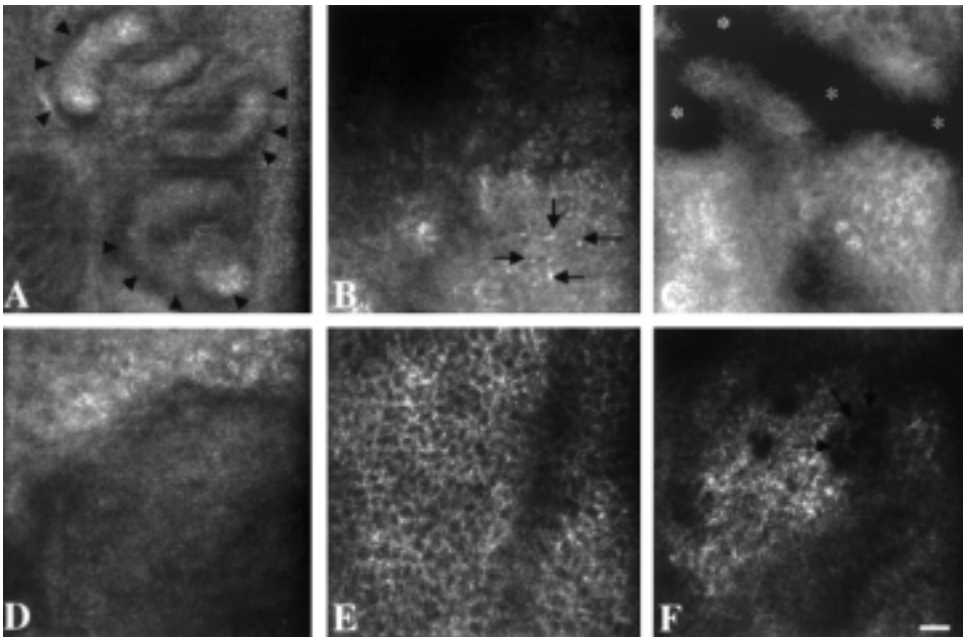

Fig 3. After pulsed dye laser exposure. **A,** Several minutes after treatment, amorphous cords of refractile material conform to shape of original vessels (*arrowheads*). **B,** One day later, discrete, bright round inflammatory cells, likely polymorphonuclear leukocytes (*arrows*), appear in epidermis and dermis. **C,** Two days after treatment, dark nonrefractile spaces 10 to 200 μm wide without blood flow, appear where vessels once were (*). **D,** One week later, cellular detail can no longer be seen in epidermis overlying center of lesion, suggesting damage to keratinocytes from vascular compromise. At 3 weeks, a normal epidermis (**E**) and dermis (**F**) containing normal caliber vessels (**F,** *arrows*) replaces treated lesion (scale bar = 25 μm).

the static images one can see individual cells within the vessels.

After PDL exposure (Fig 3)

Several minutes. Flow of blood within the vessels ceased. Instead, amorphous cords of refractile material were present, conforming to the shape of the original vessels. The epidermis appeared normal (Fig 3, *A*).

Several hours. No change was observed relative to the CM images taken after several minutes.

1 day. Amorphous, refractile cords remain within the vessels. Discrete, bright round objects smaller than 10 μm appear in the epidermis and dermis. These represent inflammatory cells, and are probably polymorphonuclear leukocytes (Fig 3, *B*).

2 days. Cords can no longer be seen within the vessels. Instead, only dark nonrefractile spaces 10 to 200 μm wide appear where the vessels once were. There was no blood flow in these spaces. The inflammatory infiltrate persists (Fig 3, *C*).

1 week. Dark nonrefractile spaces continue to occupy the dermis central to the lesion. However, growing in from the edge of the lesion are new, normal, nondilated, approximately 10 μm vessels. Cellular detail can no longer be seen in the epidermis overlying the center of the lesion, suggesting damage to keratinocytes overlying the treated vascular lesion. This damage may be because of the vascular compromise (Fig 3, *D*).

2 weeks. Central epidermal necrosis and dark dermal spaces persist, although these occur over a smaller central area with normal surrounding tissue.

3 weeks. A normal epidermis and dermis have replaced the entire lesion (Fig 3, *E, F*).

After krypton laser exposure (Fig 4)

Several minutes. Dark nonrefractile spaces of 10 to 50 μm appear where blood vessels had been. Discrete round bright objects smaller than 10 μm, representing either extravasated red cells or infiltrating inflammatory cells, appear in the epidermis and dermis (Fig 4, *A*).

Several hours. The dark nonrefractile spaces widened, up to 200 μm in diameter, and developed ragged edges (Fig 4, *B*).

1 day. Cellular detail was no longer seen in the epidermis overlying the lesion, manifesting necrosis of keratinocytes. Wide, dark spaces persist in the dermis.

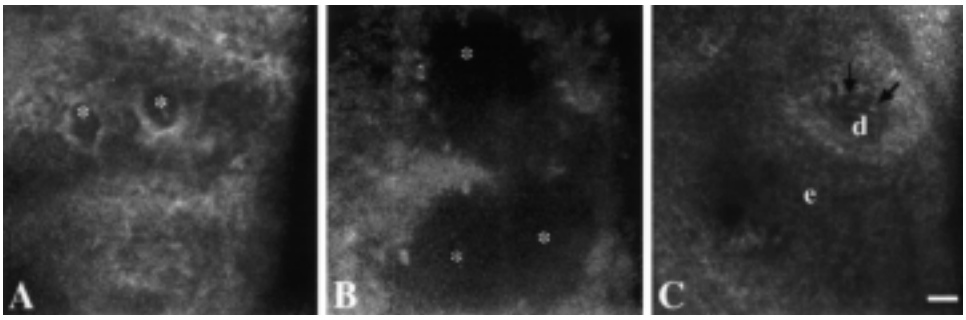

Fig 4. After krypton laser exposure. **A,** Several minutes after treatment, dark nonrefractile spaces of 10 to 50 μm appear where blood vessels had been (*). **B,** Several hours later, these spaces widen to 10 to 200 μm and develop ragged edges (*). **C,** At 4 weeks, normal epidermis (*e*) and dermis (*d*) with normal caliber vessels (*arrows*) have replaced lesion (scale bar = 25 μm).

2 days. No change is observed in the lesion.

1 week. No change is observed in the lesion.

2 weeks. Normal keratinocytes and new, normal, nondilated vessels of approximately 10 μm diameter appear at the edges of the lesion.

3 weeks. Healing continues inward.

4 weeks. A normal epidermis and dermis have replaced the entire lesion (Fig 4, *C*).

DISCUSSION

Cherry angiomas are common acquired microvascular malformations, occurring as bright red to dark purple papules up to several millimeters in size and increasing in number with age. On histologic sections, the dermal papillae each contain markedly dilated vessels, with smaller, but still dilated, vessels situated lower in the papillary dermis. Three-dimensional reconstruction of histologic sections reveals large saccular dilations of vessels within the papillae; these are connected to each other and to a normal postcapillary venule in the horizontal plexus below by a tortuous network of smaller, variable-width vascular channels.[4,5] Electron microscopic analysis of these vessels characterizes their features as those of postcapillary venules and venous capillaries.[4]

PDL operating at 585 nm is a clinically successful treatment for cherry angiomas.[6] Several studies report histologic findings after PDL treatments. Immediately after exposure, vessels within 400 to 650 μm of the skin surface are filled with cords of agglutinated, homogenized red blood cells and coagulated endothelium, molded to the shape of the original vessels. In addition, extravasated red blood cells are variably present in the upper dermis. When present, minimal epidermal damage is generally confined to the basal layer and is more prominent over superficial vessels. One day after PDL treatment, a vasculitis occurs, with endothelial cell necrosis, disappearance of the red blood cell coagulum, fibrinoid necrosis, and perivascular neutrophils, lymphocytes, histiocytes, and nuclear debris.[7-9]

Our in vivo CM of a cherry angioma treated with PDL follows a similar picture. Blood flow in the vessels was replaced within several minutes by amorphous, refractile cords conforming to the original vessel shape, representing the coagulated red blood cells and endothelium (Fig 3, *A*). Although purpura was clinically present, we could not discern any change in the perivascular tissue pattern that might represent hemorrhage. The discrete, small, bright, round objects that appeared by 24 hours represent inflammatory cells, and most likely polymorphonuclear leukocytes (Fig 3, *B*).[10] By 2 days after treatment, the intravascular coagulum and vessels were no longer seen, leaving dark spaces (Fig 3, *C*). CM images in vivo are generated from scattered light; a dark space therefore consists of material with low optical scattering, in contrast to collagen in the dermis, which is strongly scattering. The dark spaces that replaced the vessels may represent edema, fibrin, or necrosis. We were able to continue to image the lesion, observing the delayed progression of epidermal necrosis (Fig 3, *D*), because CM does not alter tissue. Moreover, we were able to observe the same lesion through neovascularization and healing to normal skin (Fig 3, *E, F*).

The krypton laser, a continuous wave laser extensively studied in the treatment of retinal vessels, is also successful in the treatment of cutaneous vessels.[11] Its 520 and 568 nm light has been applied to facial telangiectasia with good results. Unlike the PDL, which delivers a high peak power within a short pulse duration of 450 microseconds, the krypton laser delivers a continuous wave with a maximum power of about 2 W. Thus, whereas the PDL induces rapid heating, coagulation, and rupture of vessels,

the krypton laser causes slower heating and coagulation. The pattern of injury caused by krypton laser may be similar to that of the argon laser, which tends to cause nonspecific thermal injury by heat conduction to structures outside the vessels, including collagen and keratinocytes, in addition to fragmentation, clumping, and fusion of erythrocytes and endothelial cells.[12] However, krypton laser injury of skin has not been described histologically.

This in vivo CM study of laser-treated cherry angiomas shows differences between continuous-wave (krypton) and pulsed (PDL) lasers. Unlike results seen with the PDL, we saw no amorphous cords replacing the vessels after krypton laser treatment. Instead, there was immediate progression to the dark spaces that formed only 24 to 48 hours after PDL treatment. The appearance of discrete, small, round, bright cells in the surrounding dermis are most consistent with infiltrating inflammatory cells. The dark spaces widened several hours after the krypton laser treatment, and epidermal necrosis occurred in 24 hours. Within several weeks, neovascularization and healing began, completing after 4 weeks. Further study involving more lesions and other types of vascular lesions would be necessary to confirm these results because our study involved only cherry angiomas, only 2 lesions, and only 1 patient.

CM imaging is a new way to observe dynamic pathophysiologic events, such as those that occur subsequent to laser treatment of cutaneous lesions. Because the tissue is not harmed by imaging, microscopy can be performed frequently and repeatedly. CM may also be useful for measuring microvascular features that may affect response to laser treatment. For example, "resistant" port-wine stains may potentially be those with larger vessels, higher blood flow rates, or less inflammatory response after treatment.

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