Ultrastructural Changes in Human Skin After Exposure to a Pulsed Laser

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Selective vascular injury following irradiation using a pulsed laser source at 577 nm was examined using ultrastructural methods in the skin of 3 fair-skinned healthy human volunteers. This vascular-specific damage was confined to the papillary dermis. Red blood cells were altered in several ways. As well as an increase in the electron density, configurational distortion modified the normal biconcave forms to ameboid structures. The most interesting finding was the appearance within these altered cells of well-defined circular/oval electron-lucent areas of 800 Å diameter, possibly representing a heat-fixed record of steam formation within the red blood cell. In addition, considerable degenerative changes were evident in endothelial cells and pericytes, as well as collagen bundles immediately surrounding most laser-damaged blood vessels appeared normal.

It has recently been shown that suitably brief pulses from a tunable dye laser at 577 nm are selectively absorbed by the target chromophore, oxyhemoglobin [1–5], and therefore laser-induced damage can be confined to cutaneous blood vessels, particularly those in the superficial vascular plexus [6]. In these studies little or no damage to surrounding structures was seen on light microscopy. This target specificity was independent of focusing and was achieved by the choice of laser wavelength, pulsewidth, and dose. Wavelength of radiation was selected to maximize oxyhemoglobin absorption in cutaneous vessels [7]. Although the extinction coefficient of oxyhemoglobin at the 542 and 577 nm absorption bands are comparable and much less than at 418 nm, 577 nm radiation was chosen because these wavelengths penetrate deeper into tissue than either 418 or 542 nm radiation.

Theoretical calculations have predicted that it is possible to confine laser damage to blood vessels if the exposure time is significantly less than the calculated thermal relaxation time for cutaneous microvessels [8]. By varying target temperature in hamster cheek pouch [2] and human skin [4] it has been estimated that the absolute temperature achieved during laser-induced hemorrhage was near 100°C. This suggested that microvaporization may be occurring in red blood cells or blood at the time vessel rupture.

Here we report the ultrastructural aspects of selective injury to cutaneous blood vessels with the goal of obtaining information about the mechanism(s) involved.

MATERIALS AND METHODS

Subjects and Laser

Three healthy human male volunteers between the ages of 23 and 36 years with skin type I-II were exposed to varying doses of laser radiation from a Candela model LFDL 6 tunable dye laser operating at 577 nm using a rhodamine 575 dye at a pulsewidth of 1.5 µs. To avoid known competitive absorption of photon by melanin at this wavelength [6], all volunteers were individuals with minimal skin pigmentation. The laser was focused using a planoconvex lens into a quartz optical fiber through a field lens system. Laser pulse energies were measured using a Scientek model 362 laser energy meter calibrated to ±5% accuracy. Incident energy densities were calculated based on the diameter of the circular field of irradiation. Separate 2 mm-diameter sites were exposed to single laser pulses at doses ranging from 0.25–3.0 J/cm² in 0.25 J/cm² increments.

Clinical (morphologic) threshold was arbitrarily defined as the presence of nonblanchable purpura filling the whole spot size or irradiated site of 2-mm diameter. The energy required to produce this effect was defined as the threshold dose.

Three-millimeter punch biopsies were taken at threshold dose after infiltration of the skin with 1% Xylocaine in all 3 subjects. In 1 subject, 2 additional biopsies were taken at doses of 0.25 and 0.5 J/cm² above threshold. All skin biopsies were obtained within 10 min of laser irradiation.

Tissue Preparation

The tissue was fixed in a mixture of 2.5% glutaraldehyde and 2% paraformaldehyde solution at room temperature for 2 h and spot-fixed in 2% osmium tetroxide at 4°C for 2 h. All fixatives were diluted in 0.1 M cacodylate buffer at pH 7.4. The fixed tissue was then dehydrated in a graded ethanol series, passed through propylene oxide, and embedded in Epon 812. Ultrathin sections were cut serially with diamond knives on a Porter-Blum MT-2 microtome, counterstained with uranyl acetate and lead acetate solutions, and examined with a Zeiss EM 109 electron microscope operating at 80 kV.

RESULTS

Light microscopy confirmed earlier studies which showed that, under the laser exposure conditions used here, injury was confined to cutaneous blood vessels, especially those in the papillary dermis, to a depth of about 0.2 mm from the dermo-epidermal junction. The superficial vascular plexus as well as the ascending and descending limbs of the capillary loop vessels were the most vulnerable. However, this effect was not uniformly distributed and occasional less severely affected microvessels were also seen, especially in the upper reticular dermis.

One-micron sections and low-magnification electron microscopy of skin at threshold dose revealed hemorrhage, intravascular erythrocyte aggregation within the vessel lumen, and blood vessel wall damage including vessel rupture in some instances. Typical microscopic changes seen at laser threshold doses are shown in Fig 1A,B. A postcapillary venule is seen in Fig 1A, an ascending arteriolar and venous limb of the capillary loop are shown in Fig 1B. Within the same field in Fig 1A is a mast cell, a few fibroblasts, histiocytes, lymphocytes, and several extravasated red blood cells. Clumps of irregularly distributed electron-dense material giving the impression of streaming at the periphery and occasionally intermingled with fibrin strands (Fig 2A) were almost always seen within the lumen of both damaged arteriole and venule (Fig 1B). The limiting membrane is hard to discern in most instances (Fig 2B). However, when blood vessels were severely damaged by laser irradiation, as occurred at suprathreshold doses and when blood
vessel walls actually ruptured, this material traversed possible breaks in vessel walls and was found outside blood vessels intermingled with fibrin strands. Also present within the lumen were mature neutrophils, some normal-appearing red blood cells, fibrin strands, and platelets (Fig 1A,B). Most of the neutrophils appeared to be intact, with an occasional cell actively engaged in phagocytosis of this electron-dense material.

Red blood cells were altered in several ways by the tunable dye laser at 577 nm. There was an increase in the electron density of the red blood cells within the exposed field compared to normal red blood cells. The configuration of these cells varied from being the normal biconcave to ameboid-like structures. Microspherules were present, apparently formed by “budding” from red blood cells. A most interesting finding was the presence of a large number of well-defined, circular and oval electron-lucent areas (average diameter of 800 Å), which were scattered in this electron-dense material (Fig 2B). Some of these lucent circles contained flocculent material. Normal unirradiated control skin biopsy processed under exactly similar conditions did not show any of these changes.

Endothelial cells and pericytes (or smooth muscle cells) revealed considerable degenerative change manifested by nuclear pyknosis, cytoplasmic vacuolization, mitochondrial de-

![Figure 1](image1.png)

**FIG 1.** A, Low-power electron micrograph at threshold exposure doses showing a postcapillary venule (encircled by arrowheads), clumps of irregularly distributed electron-dense material (asterisk), extravasated red blood cells (white dots), and dermal cells including fibroblasts, histiocytes, and mast cells. Bar = 10μm. B, Electron micrograph at threshold exposure doses showing the damaged ascending limb of the capillary loop (right). Intravascular erythrocyte aggregation is present within the lumen of the venous limb (left). Compare the electron density of clumps of irregularly distributed materials (asterisks) with that of normal-appearing red blood cells (R). Bar = 5μm.

![Figure 2](image2.png)

**FIG 2.** A, High-power view of the clumps of electron-dense material described in Fig 1A. It is intermingled with fibrin strands (F). N = Neutrophils. Bar = 1μm. B, High magnification of the electron-dense material with numerous circular and ovoid electron-lucent areas. It lacks a limiting membrane. Note the presence of osmiophilic limiting membranes of a neutrophil and a normal red blood cell (inset, arrows). Bar = 1μm.

![Figure 3](image3.png)

**FIG 3.** Endothelial cell (E) of the postcapillary venule. Note nuclear pyknosis, cystic dilatation of rough endoplasmic reticulum (asterisk), and vacuolated nuclear membrane (arrowhead). Arrows point out the multilayered basal lamina. N = Neutrophil. Bar = 1μm.
generation, and cystic dilatation of rough endoplasmic reticulum (Fig 3). In most instances, the endothelial cell nuclear membranes were vacuolated and cytoplasmic membranes were difficult to delineate. In some areas, only ghostlike remnants of endothelial cells were present. Ultrastructural changes seen in pericytes (or smooth muscle cells) were similar to those observed and described in endothelial cells but were, in general, more severe despite being anatomically a greater distance from the blood vessel lumen than endothelial cells (Fig 4). This suggests that pericytes (or smooth muscle cells) may be more susceptible to laser-induced damage at this wavelength. Occasional breaks in the endothelial lining were also noted (Fig 5A). As well as this, occasional separation of the basal lamina was seen. Red blood cells were often found lying within the basal lamina, distorting its configuration (Fig 4). Scattered either between the layers of basal lamina or lying outside the damaged blood vessels were vacuolated, membrane-bound structures with flocculent material within them (Fig 5B).

Collagen bundles and elastic fibers immediately surrounding laser-damaged blood vessels appeared to be ultrastructurally intact and normal. As well as these, collagen bundles, which were sparsely distributed between layers of basal lamina, especially in veins, preserved their periodicity.

In general, at threshold doses, neurons, mast cells, histiocytes, and fibroblasts surrounding laser-damaged blood vessels were intact. Only at suprathreshold doses when severe vessel damage was evident did nearby fibroblasts show some degenerative changes as manifested by nuclear pyknosis, cytoplasmic vacuolization, and mitochondrial swelling. Even at these higher doses mast cells remained intact and no evidence of degranulation was detected.

Focal epidermal changes were evident in 2 of the 3 subjects investigated. This consisted of cytoplasmic degeneration of basal keratinocytes and melanocytes (Fig 6). At threshold doses, vacuolization of keratinocytes was seen in areas immediately adjacent to melanocytes. At suprathreshold doses, the vacuolization was enhanced especially in the area of the perikaryon, and melanocytes showed swollen mitochondria with the loss of cisternae (Fig 7). Again, control skin biopsy specimens processed under exactly similar conditions did not show any of these changes.
DISCUSSION

This study confirms that it is indeed possible to confine laser damage to selected sites in the skin by the appropriate choice of a wavelength, pulsewidth, and dose. 

The most striking laser-induced changes observed in this study were those seen in red blood cells and cellular structures in blood vessel walls surrounding these abnormal erythrocytes. Although many studies have shown morphologically abnormal spheroid red blood cells after thermal injury [9,10] as well as the formation of intravascular thrombi after laser irradiation [11-13], we are not aware of any studies describing the type of red blood cell changes seen here. The laser-induced thrombi described in some of these studies consisted mainly of either aggregated platelets [12], white blood cells [13], erythrocytes [11], or a combination of these cellular components, but in none of these studies were changes of an increase in electron density and an alteration of the shape of red blood cells from being normal biconcave to ameboid-like structures observed or described. We propose that these changes are probably the result of rapid, intense, localized, thermal injury of red blood cells. In some instances, thermal alteration of the red blood cells within the vessel lumen was focal and normal erythrocytes were seen immediately adjacent to these abnormal structures. The presence of an admixture of thermally altered and normal red blood cells within a vessel lumen could be the result of the reestablishment of blood flow to the area in the interval between laser irradiation and the time the punch biopsy was taken.

However, of most interest is the presence of circular and oval electronlucent areas scattered within the substance of the altered erythrocytes. We propose that these electron-lucent areas may be a “heat-fixed” permanent record of steam formation within the red blood cells. During steam formation in the red blood cells, denaturation of hemoglobin and structural proteins adjacent to the steam may lead to temporary phase changes which accomodate steam-induced volume expansion. Upon cooling, reversal of this phase change could leave a mold or cast of the steam bubble. Steam itself could certainly not be present at the time of biopsy (up to 10 min later) or survive fixation but its earlier presence is recorded by the denatured material around the former bubbles. Goldman et al [14] reported the presence of “steam bubble” areas within thermally damaged human skin using a Q-switched ruby laser but did not specify the exact size, site, or nature of these structures and they may have represented edema fluid.

It has been calculated in other studies [2,4] that peak temperatures of 80-100°C were reached in blood vessels during irradiation using the tunable dye laser at 577 nm at a pulse duration of 300 ns. These temperatures are well above those at which proteins denature, enzymes degrade, and red blood cells alter in shape, which are all known to take place at around 50°C. At this temperature, erythrocytes alter in shape by either crenating or becoming spheroid and are more fragile [9,10]. The Arrhenius model [15], an empirically derived mathematical description of first order temperature-dependent chemical rate processes, holds for denaturation except for modification by sharp transitions due to intramolecular cooperativity. Because the amount of denaturation depends in part on the total number of molecular collisions, and therefore on time-temperature history, one would predict that the maximum temperature which must be achieved for denaturation to occur would increase as duration of heating decreases. For heating by light pulses shorter than 10^-6 s, the required temperatures may be in excess of 100°C so that the first event upon rapid heating may be phase change in solvent and not denaturation. In this study, therefore, steam formation during peak temperature rise may precede denaturation which occurs during cooling of the target (red blood cells).

Structures such as endothelial cells and pericytes which immediately surround these erythrocytes were also extensively damaged. Such marked changes of cellular components of blood vessel walls have not previously been reported after laser-induced microvascular irradiation [11,12]. In contrast to the severe damage seen in these specific structures, mast cells, histiocytes, and fibroblasts immediately in the vicinity of these laser-altered structures were essentially intact. Even collagen fibers lying around these vessels appeared normal and retained their periodicity and only minimal alterations were seen in dermal nerves. Mast cells have been shown to degranulate at temperatures as low as 54°C [16], and Gorisch and Boergen [17] also clearly demonstrated that collagen fibrils in blood vessels showed evidence of denaturation at around 70-75°C.

Another interesting observation made in this study was the presence of spheroid erythrocytes lying mainly within the lumen of laser-damaged vessels and, occasionally, outside, which blood vessels ruptured. Fibrin strands are apparently not commonly seen ultrastructurally in blood except when it is altered in some way like prolonged storage for more than 1 week [18]; neither have they been routinely found after laser irradiation [11]. However, previous studies have shown that temperature affects the formation of fibrin complexes [19]. Edgar and Prentice [19] showed that the actual structure of these complexes was altered at a temperature of 37°C and this in turn, affected the stability of the soluble fibrin complexes. Therefore, it is conceivable that fibrin strands seen in our specimens are the result of the thermal alteration of these complexes.

At the present time, it is unclear why certain structures surrounding laser-targeted red blood cells like pericytes (or smooth muscle cells) and endothelial cells are more susceptible to laser damage at 577 nm than are other thermally vulnerable cells like mast cells which have been shown to alter histochemically as early as 15 min after thermal injury [20]. Further studies are being planned to examine this effect.

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REFERENCES