Chapter 4

Histologic evaluation of skin damage after overlapping and non-overlapping flashlamp pumped pulsed dye laser pulses; a study on normal human skin as a model for portwine stains

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Abstract

Background and objectives: The treatment of burn wound scars with the flashlamp-pumped dye laser is a well-known technique. However, the use of multiple, sequentially delivered pulses with decreasing temporal separation has been proposed as a method for improving scar appearance. In this report, we investigate the effects of sequentially delivered pulses on scar appearance.

Methods: Materials and Methods: The study consisted of 40 patients with burn scars. The patients were randomized to receive one of four treatment protocols: (1) single pulse, (2) two pulse, (3) three pulse, or (4) four pulse. The scar appearance was assessed using a blinded, randomized, controlled trial.

Results: The sequential delivery of pulses resulted in a significant improvement in scar appearance. The improvement was statistically significant compared to the single pulse treatment. The improvement was greatest with the four pulse treatment.

Conclusions: Sequential delivery of pulses with decreasing temporal separation may be a more effective method for improving scar appearance. Further studies are needed to determine the optimal pulse parameters and the long-term effects of sequential pulse treatment.
Introduction

The flashlamp pumped pulsed dye laser is widely accepted as the laser of choice for treatment of portwine stains, especially when treating children.\(^1\) Wavelength and pulse duration of the flashlamp pumped pulsed dye laser are fixed and the spot size is limited to 5-10 mm diameter (depending on the laser model); in order to treat an area of the portwine stain as large as possible the maximum spot size will usually be selected. A parameter that can be varied is the radiant exposure (J/cm\(^2\)) of the pulse. Previous studies have investigated threshold levels; radiant exposure levels lower than 4 J/cm\(^2\) do not seem to have clinical relevance.\(^7\) Treatment practice learns that mostly radiant exposure levels between 5 and 8 J/cm\(^2\) are used.

No consensus exists on how to place the pulses: in an overlapping or non-overlapping way. Dinehart et al. recommend overlap of exposure spots based on beam profile measurements showing central peaking of energy.\(^8\) Clinically, the aim is to achieve homogeneous lightening of the portwine stain. To prevent the occurrence of a reticular pattern, which is inevitable when pulses do not overlap, overlapping pulses are clinically preferable. It even seems that the reticular pattern that arises after treatment with non-overlapping pulses is more difficult to treat than the original portwine stain (O.T. Tan, Boston, personal communication). Theoretically, there is an increased risk of redundant tissue damage with overlapping pulses, since the overlapping laser pulse is partially aimed at a changed target. The vessels in this target area which have already been damaged, will be irradiated again after a short time. Thus far, little information is available about the extent of resulting dermal and epidermal damage after application of overlapping pulsed dye laser pulses with clinically relevant radiant exposure levels.\(^9\)

The aim of this study was to establish the histopathological effects of overlapping laser pulses on the skin and in particular on the dermal vascular plexus. Since at least four biopsies per individual had to be taken, the study was performed on healthy skin of pre-operative volunteers instead of actual portwine stain skin. The relevance of our
findings in normal human skin or course of a standard treatment will be
addressed in the discussion.

**Materials and methods**

**Study design**

A series of experiments were conducted on normal human skin
by the Transverse Technique. The level of skin perfusion, measured
as the area of skin heated by laser light, was followed in rabbits by
repeatedly putting two laser pulses with increasing radiant
exposure.

Another study using both pulse exposures was performed using the
same exposure rates.

**Patients**

Fifteen healthy Caucasian women who were hospitalized for breast
reduction surgery (30 breasts) underwent skin perfusion and are
ticipated to participate in the study. Eight of the women who underwent breast
reduction agreed to participate in our study. A number of breasts was
exposed to two laser pulses with increasing radiant exposure, and 31 breasts
were exposed to two laser pulses with different degrees of overlap. The 2
breasts were followed for 1 year, and all patients participated for the
steroid exposure.

**Increasing radiant exposure**

The radiant exposure was varied with a Nd:YAG laser system manufactured by
ucumber Medical Systems, Santa Clara, CA, USA. The pulse length of the laser was set at 3 ns
and the output was 15 mJ per pulse in a minimum diameter of 1 mm, ranging to 3 mm.

In addition to using the Transverse Technique, we repeated the pulses
from the second laser run of the rate of radiant exposure was
analysed. The data for exposure rate was counted to ensure that the
exposure was the same for each of the skin perfusion.

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Fig. 1: Schematic overview of pulse sites.

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Immediately after removal of the skin and subcutaneous tissue, the reconstructive surgical procedure for the exposed sites was carried out. The exposed sites were covered with a dressing, and the dressing was changed every 24 hours. After closure, the skin and subcutaneous tissue were cut to form a flaps. The flaps were transferred to the wound, and the wound was closed. We also performed additional operations, including the transfer of the skin and subcutaneous tissue. In the experiments, we looked for evidence of self-healing and the formation of new blood vessels. Experimental results showed that the transfer of the skin and subcutaneous tissue was successful with the use of Zoss equipment. We also transferred vessels included the artery and vein, and the vessels were reanastomosed with the vessels.
Results

Effect of increasing radiant exposure level
Macroscopically, purpura appeared within a few seconds after laser exposure, turning from grey to purple in the next 24 hours. The higher the radiant exposure level the darker the spot became. With energy levels of 9 and 10 J/cm² blisters were sometimes seen. Apart from these cases, patients did not notice any systemic or substantial local discomfort (usually only a slight itch at the pulse sites). Diameters of skin purpura were invariably 4 mm, irrespective of radiant exposure level.

Fig. 2: Deepest vascular damage, averaged over all patients, as a function of radiant exposure level. The error bars indicate 95 percent confidence intervals.
Fig. 2 shows that the depth of deepest vascular damage is between 1.5 and 2 mm for 6, 7 and 8 J/cm$^2$. With 9 and 10 J/cm$^2$ depths of over 2 mm were accomplished. However, histopathologically we observed that with these radiant exposure levels often epidermal damage occurred (vacuolization, necrosis, Fig. 3a), and in a number of cases also damage of adnexal structures or even coagulative changes of dermal collagen (Fig. 3b and Fig. 4). Since this might result in scarring and/or pigmentary changes we decided that the radiant exposure used for the overlap experiment should not exceed 8 J/cm$^2$. Pulses with radiant exposure of 6, 7 and 8 J/cm$^2$ seem to result in comparable depths of vascular damage. We therefore selected 7 J/cm$^2$ for the overlapping pulses experiment.

![Laser exposed skin, radiant exposure 10 J/cm$^2$. The epidermis shows areas of coagulative epithelial necrosis (pale staining areas, indicated by arrows). H&E stain, 25x.](image-url)
Fig. 3b: Detail of the dermis with perivascular infiltration of inflammatory cells, mostly neutrophils, depositions of nuclear dust (leucocytoclasia) and massive extravasation of erythrocytes. Vessel walls are completely destroyed and have almost disappeared (see arrows). H&E stain, 160x.
Fig. 4: Detail of the dermis, exposed to 10 J/cm², both photographs are taken at the same magnification. Panel A: the dermal collagen structure of the laser exposed skin reveals that the diameter of fibers is slightly decreased, the fibers stain darker (due to increased eosinophilia) and are more homogeneous in structure (due to loss of the fine fibrillar pattern). These alterations indicate coagulative changes of the exposed dermal collagen. For comparison a detail of the normal adjacent (non exposed) reticular dermis is shown (panel B). H&E stain, 245x.
Effect of overlap

Diameters of skin purpura after the various types of exposure (after formalin fixation) were as follows: single shot 4 mm; total overlap 4 mm; 30 percent overlap 7 mm; separated pulses both 4 mm. Histologically, skin damage was almost exclusively confined to the dermal blood vessels, irrespective of the type of exposure. Abundant vessel necrosis and leukocytic infiltration were seen. The surface area of vessel damage corresponded with the macroscopic diameter of the purpura. The depth of vascular damage varied with the type of exposure, being most extensive for overlapping pulses (total and 30 percent overlap), followed by separated and single pulses respectively (Table 1). The two values in the last column represent the depths of vascular damage of each of the two separated pulses. Within each individual the separated pulses showed a high and a low value for the depth of vascular damage. In the last column of Table 1 these were labeled ‘low’ and ‘high’. We did not mark the two separated pulses as first and second, but it seems plausible to relate the lowest value to the first pulse and the highest value to the second pulse. We have also considered variability in depth of vascular damage between pulses within one patient as an explanation for the differences in depths. However, this seems unlikely because of the similarity in depth of vascular damage in each patient between a) the single pulse and the lowest value of the separated pulses, and b) the overlapping pulses and the highest value of the separated pulses.
Table 1: Depth of vascular damage (in mm) for all different types of exposure at 7 J/cm².

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>single shot</th>
<th>total overlap</th>
<th>30 percent overlap</th>
<th>separated pulses low &amp; high</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 breast</td>
<td>0</td>
<td>21</td>
<td>24</td>
<td>17 &amp; 22</td>
</tr>
<tr>
<td>2 breast</td>
<td>10</td>
<td>22</td>
<td>26</td>
<td>18 &amp; 24</td>
</tr>
<tr>
<td>3 breast</td>
<td>13</td>
<td>22</td>
<td>22</td>
<td>16 &amp; 23</td>
</tr>
<tr>
<td>4 breast</td>
<td>18</td>
<td>22</td>
<td>18</td>
<td>14 &amp; 18</td>
</tr>
<tr>
<td>5 breast</td>
<td>20</td>
<td>22</td>
<td>15</td>
<td>18 &amp; 28</td>
</tr>
<tr>
<td>6 breast</td>
<td>23</td>
<td>22</td>
<td>22</td>
<td>28 &amp; 30</td>
</tr>
<tr>
<td>calf</td>
<td>2</td>
<td>18</td>
<td>18</td>
<td>16 &amp; 18</td>
</tr>
<tr>
<td>ankle</td>
<td>2</td>
<td>18</td>
<td>18</td>
<td>19 &amp; 22</td>
</tr>
<tr>
<td>malleolar</td>
<td>12</td>
<td>18</td>
<td>18</td>
<td>12 &amp; 14</td>
</tr>
</tbody>
</table>

In many cases the level of deepest vascular damage was found at sites where vessels accompanied adnexal structures (Fig. 5a). But not, the deepest level was always in the center of the lesion, including the 30 percent overlap pulses.

Vessels in the center of the lesion site were always most severely damaged, showing complete destruction necrosis and fragmentation of the vessel wall, and phagocytosis of neumat with thrombus. The perivascular stroma contained extravasated erythrocytes and an inflammatory infiltrate composed of granulocytes, partially fragmented, and to a lesser extent mononuclears and eosinophils (Fig. 5b). Towards the periphery of the lesion the vessel walls were not necrotic but showed marked endothelial swelling, perivascular infiltrate and permeability for erythrocytes; these changes reached up to 3 mm from the DLJ, sometimes extending into the subcutaneous fat, but were not included in the measurements of vessel damage. There was no destruction of the dermal...
connective tissues (collagen and elastin), only a slight perivascular oedema and focal fat necrosis around adnexal vessels were sometimes seen.

Fig. 5a: Overview of a laser exposed skin site, 30 percent overlapping pulses, radiant exposure 7 J/cm², showing infiltrates of inflammatory cells in the upper reticular dermis, extending into the deeper dermis along adnexal structures. Extravasated erythrocytes, present in the sections, are not visible at this magnification. H&E stain, 30x.

There was a marked difference in the extent of inflammation and bleeding between individuals. Moreover, breast skin lesions were deeper and showed more inflammation than abdominal skin lesions. This can not be explained by the slight difference we found between epidermal thickness of breast skin (0.025-0.05 mm) and abdominal skin (0.015-0.03). Neither was there a visible difference in melanin content between breasts and abdomens. There were no obvious differences in inflammatory response between single and overlapping pulses in one and the same individual.
Fig. 5b: Detail of the dermis. In the center a dilated vessel (venule) with destruction of the vessel wall and plugged with erythrocytes and platelets. Perivascular oedema, inflammatory cells and extravasation of erythrocytes. H&E stain, 240x.

To identify the effects of different pulse applications in spite of differences between patients, we further analyzed the data of Table 1 using paired two-sided t tests. This approach should correct for the influence of differences in depth of vascular damage between individual patients. A p-value <0.05 means that the two compared variables are significantly different from each other.
Table 2: Results of the paired two-sided t test; \( p < 0.05 \) means that the compared variables are significantly different from each other.

<table>
<thead>
<tr>
<th>compared variables</th>
<th>difference in depth of vascular damage*</th>
<th>95 percent confidence interval</th>
<th>( p )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>total overlap &amp; single pulse</td>
<td>0.42</td>
<td>(0.37, 0.85)</td>
<td>0.0003</td>
</tr>
<tr>
<td>30 percent overlap &amp; single pulse</td>
<td>0.48</td>
<td>(0.23, 0.73)</td>
<td>0.0019</td>
</tr>
<tr>
<td>total overlap &amp; 30 percent overlap separated pulses: high &amp; low</td>
<td>0.13</td>
<td>(-0.05, 0.31)</td>
<td>0.13</td>
</tr>
<tr>
<td>separated low &amp; single pulse</td>
<td>0.17</td>
<td>(-0.12, 0.46)</td>
<td>0.22</td>
</tr>
<tr>
<td>total overlap &amp; separated high</td>
<td>0.04</td>
<td>(-0.16, 0.24)</td>
<td>0.66</td>
</tr>
</tbody>
</table>

* difference per patient, averaged over the ten subjects.

In Table 2 the results of the analysis are summarized:

1. Overlapping pulses cause vascular damage to a significantly deeper level than single pulses. This is true both for total overlap as well as for 30 percent overlapping pulses.

2. There is no significant difference between depth of vascular damage between totally overlapping and partially (30 percent) overlapping pulses.

3. When pulses are separated but close together there is a significant difference in depth of vascular damage between the two.

4. The lowest value in depth of vascular damage of the two separated pulses (see last column of Table 1) is comparable to the depth of vascular damage of the single pulse in the same patient; the highest value is similar to the depth of vascular damage caused by overlapping pulses.
Discussion

The aim of this study was to identify possible differences in skin damage between overlapping and non-overlapping flashlamp pumped pulsed dye laser pulses on human skin, with normal skin as a model for portwine stain skin.

We found that in normal skin separated pulses do not behave as two independent single pulses. Somehow, a laser pulse affects the skin in such a way that a second pulse, whether overlapping the previous one or not, causes significantly deeper dermal vascular damage than one single pulse.

Considering the rapidity of this phenomenon, it must be caused by a fast propagating mechanism, reaching a distance of at least 5 mm within 3 seconds. Heat conduction is probably too slow, although convective heat transport by blood flow may be fast enough. It is also possible that vessels in the periphery of the injured tissue-volume are damaged but not completely destroyed, and the remaining blood flow transports vaso-active components to the surrounding tissue. A fast propagating mechanism is the shockwave produced by the 0.45 ms pulse. In a direct or indirect way (e.g. by neuronal pathways), this probably causes a vaso-active reaction. Although we have not identified the precise mechanism, we hypothesize that as a result of all these possibly concurrent phenomena, reactive hyperaemia is induced at the site of incidence of the second pulse. This would increase the number of erythrocyte-filled capillaries in normal skin, resulting in a deeper level of vascular damage after the second laser pulse. It is also possible that the reason for the second pulse effect in normal skin, portwine stain skin may react differently. In portwine stain skin all vessels are already wide open and completely filled with erythrocytes and reactive hyperaemia will not alter the number of target vessels. The difference in depth between first and second pulses may therefore not occur in portwine stain.

Nevertheless, we expect the after effects observed to be similar in portwine stain and normal skin for a number of reasons:

- Despite differences in vessel size and red blood cell concentration the
mechanism of injury in both normal and portwine stain skin is vessel
destruction by heating of red blood cells.
- the radiant exposure level of 7 J/cm² we applied for normal human
skin in this study, is in the range clinically used for portwine stain
treatment.6
- comparing the depth of vascular damage we found in normal human
skin with results reported in portwine stains,3 the laser injured skin
volumes are similar, i.e. a disk-shape of 5-10 mm in diameter (defined
by the laser spotsize) and 1-2 mm in depth.
Therefore, thermal energy deposition and type of tissue injury will be
comparable for normal and portwine stain skin.
The newer models flashlamp pumped pulsed dye lasers that are available
nowadays have a higher pulse repetition rate and larger spot sizes than
the laser used for our study. Whether the results using a repetition rate of
1 Hz and a 10 mm spot size will be comparable to our results requires
further investigation.
Our results indicate that in normal skin scarring is unlikely after
overlapping pulses with a radiant exposure level of 7.0 J/cm². In normal
skin there were no signs of coagulation necrosis of the dermal connective
tissues, whether pulses did or did not overlap. Some degree of connective
tissue breakdown and remodelling in the later stages of tissue repair may
be anticipated since the granulocytes which dominate the infiltrates in the
early healing phase are able to secrete a broad repertoire of lytic enzymes.
However, to evaluate this, consecutive biopsies taken up to a period of
three weeks after the laser exposure are required, which is obviously
difficult to realize in an experimental setting using human volunteers.
Nevertheless, histologically the pathologic features closely resemble a
leucocytoclastic vasculitis which is usually not associated with the
formation of significant fibrosis.
As we hypothesized in the first part of the discussion we expect this to be
true for portwine stains as well. The observations in this study also
correlate with our clinical experience in portwine stain patients. In a
previously reported study we treated 100 patients with facial portwine
stains (ages from 0 to 50 yr) with overlapping flashlamp pumped pulsed
dye laser pulses, at radiant exposures levels from 6 to 8 J/cm². No scarring was observed during a three year follow up period. We conclude that it is safe to treat portwine stains with overlapping flashlamp-pumped pulsed dye laser pulses in order to achieve homogeneous lightening of the stain.

References


