Histological study on the treatment of vascular malformations resistant to pulsed dye laser

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Background and Aims: The pulsed dye laser (PDL) is recognized both as an effective treatment for vascular malformations and the as first treatment of choice for these lesions. However, PDL irradiation has poor efficacy in some patients, particularly the elderly. The present study histologically assessed such patients to try to elucidate the reason.

Materials and Methods: A pulsed dye laser was used in 3 subjects in whom previous laser treatment was ineffective. Three-millimeter punch biopsies were obtained before laser treatment, 1 week and 3 months after the laser treatment. Each specimen was stained with toluidine blue and examined under light microscopy followed by electron microscopy with oolong tea extract (OTE) staining.

Results: Microscopy revealed an increase in the vasculature at baseline and an increased number of dermal fibroblasts. One week post-irradiation, inflammatory cell infiltration was observed together with extensive interstitial perivascular edema. At 1 week and 3 months after laser irradiation, normal structures were observed for both blood vessels and capillary endothelial cells. Mild changes were noted in other interstitial features, but findings obtained 3 months after irradiation were almost similar to those before irradiation.

Conclusions: The lower efficacy of PDL treatment in the elderly was possibly due to the markedly low amount of red blood cells in our subjects’ blood vessels, a major chromophore for the PDL, which was markedly low. It is possible that age-related denaturation of dermal matrix collagen plays some role in maintaining the vasculature in the interstitium with edema, and inflammatory cell infiltration could lead to the cellular release of some cytokines which favor reconstruction of the vasculature.
received radiotherapy, and 1 patient had had dry ice or liquid nitrogen therapy and radiotherapy, more than 40 years previously, but precise details of these therapeutic strategies were unavailable. In addition, all 3 patients reported that they had received laser treatment more than 10 years ago, but that they were unaware as to which specific laser device had been used, and that there were no obvious effects. All patients visited our hospital to receive laser treatment again. Laser treatment was performed using a pulsed dye laser device (Chromogenex VIII, Chromogenex, UK; 585 nm, pulse width 300 µsec, spot size 5 mm, fluence 7 J/cm²). During the treatment course, skin biopsies were taken for histological examination as described below.

**Morphological Assessment**

Three millimeter punch biopsies were obtained before laser treatment, 1 week after the laser treatment, and 3 month after the laser treatment. Each specimen was fixed in glutaraldehyde (2.5%) and then in osmium tetroxide (1%). After dehydration through a graded ethanol series, the specimens were embedded in Epon 812 (Oken Shoji Co., Ltd, Tokyo, Japan), stained with toluidine blue, and examined with light microscopy. Ultrathin sections were prepared with an Ultracut N ultramicrotome (Reichert-Nissei, Tokyo, Japan) and a diamond knife. Sections were stained with oolong tea extract (OTE) for connective tissue, and with uranyl acetate and lead citrate prior to electron-microscopic examination (75 kV, Hitachi H-7500, Hitachi, Tokyo, Japan).

**Results**

Since the 3 patients examined in this study showed similar histological findings at each of the sampling times, the findings common to the 3 patients are described below.

**Light microscopic observation (toluidine blue stain)**

Before laser irradiation (Figure 2a), the structure of the epidermis (E) was almost normal. There was proliferation of slightly dilated blood vessels and fibroblasts in the dermis (D). One week after laser irradiation (Figure 2b), interstitial edema was seen around the capillaries. Three month after laser irradiation (Figure 2c), the edema in the dermis, which was observed 1 week after irradiation, had disappeared. The density of capillaries in the dermis was reduced compared to before laser irradiation, but there was still a high proliferation of blood vessels and fibroblasts, with the former demonstrating mild dilation.

**Ultrastructural observation**

1) Before laser exposure

There was an increase in the number of capillaries, as shown by light microscopy. However, the structure of capillaries (CAP), fibroblasts (FB) and mast cells (M) appeared normal, and the dermal interstitium was filled with collagen fibrils (Co) (Figure 3a). In the interstitium, elastin-free elastic fibers (EF) were observed and spiral collagen (arrow) was present in normally structured collagen fibrils (Figure 3b).

2) 1 week after laser exposure

Extensive edema was seen around the capillaries (Cap), and inflammatory cell infiltration with fibroblasts (FB), mast cells (MC) and lymphocytes (L) was observed around blood vessels (Figure 4a). The structure of capillary endothelial cells was normal, but the arrangement of collagen fibrils was disturbed around the capillaries and there was a small amount of spiral collagen (arrow) (Figure 4b).
Figure 2: Light microscopic findings in toluidine blue-stained specimens.
Before laser exposure (Figure 2a), the epidermis (E) appears almost normal. Proliferation of slightly dilated blood vessels and fibroblasts is observed in the dermis (D). One week after laser exposure (Figure 2b), there is interstitial edema around the capillaries. Three months after laser exposure (Figure 2c), the edema in the dermis, which was apparent 1 week after exposure, has disappeared. Although dermal capillaries are reduced compared to before laser exposure, many endothelial cells can still be observed in the dermis that have not yet formed obvious lumens.

Figure 3: Ultrastructural observation before laser exposure
The number of capillaries has increased, as shown by light microscopy. However, capillaries (CAP), fibroblasts (FB) and mast cells (M) show normal architecture, and the dermal interstitium is filled with collagen fibrils (Co) (Figure 3a). In the interstitium, elastin-free elastic fibers (EF) are observed and spiral collagen (arrow) can be seen in normally structured collagen fibrils (Figure 3b).

Figure 4: Ultrastructural observation 1 week after laser exposure
Extensive edema is observed around the capillaries (Cap), and inflammatory cell infiltration with fibroblasts (FB), mast cells (MC) and lymphocytes (L) is evident around the blood vessels (Figure 4a). Although capillary endothelial cells have a normal structure, collagen fibril arrangement is disturbed around the capillaries and a small amount of spiral collagen (arrow) can be observed (Figure 4b).
3) 3 months after laser exposure

Interstitial edema around the capillaries (Cap) had disappeared. Normally structured collagen fibrils were mixed with many spiral collagen fibrils (arrow) (Figure 5a). The structure of capillary endothelial cells was normal, as observed 1 week after irradiation, but many spiral collagen fibrils (arrow) and elastin-free elastic fibers (Ef) consisting of only elastic filaments were observed around the capillaries (Figure 5b).

Discussion

The pulsed dye laser (PDL) in the yellow waveband at 580 – 590 nm and the Nd:YAG laser at the wavelength of 1064 nm are mainly used in the laser treatment of vascular malformations and, in particular, PDL is highly indicated for superficial blood vessels. On the other hand, PDL treatment is less effective in some cases of vascular malformation. For example, some pediatric patients who received laser treatment show vascular proliferation, in which the proliferation of the endothelial cells is seen accompanied by the formation of anastomotic channels filled with blood. It has also histologically been demonstrated that laser treatment has a weak effect on lesions which are composed mainly of small vessels in the deep dermis. In addition, it was reported that laser treatment is more effective for the type of lesion consisting of vascular ectasia localized in the capillary loops than for that consisting of dilated ecstatic vessels in the superficial horizontal plexus in a ring pattern. It has been hypothesized that the local action of some vascular endothelial growth factors may cause such differences in the effect of laser treatment, but this has not yet been verified.

It is said that children show a poor response to laser treatment because the nature of the vasculature of children is cellular. It has also often been our experience that laser treatment is less effective in some elderly patients. Combination treatment with PDL and Nd:YAG laser, and so on, have often been attempted, and different protocols with PDL, such as the PDL double-pass treatment, have also been tried in such patients. In addition, local steroid injection and liquid nitrogen cryopexy are also used in pediatric patients with endothelial cell proliferation.

In this study, we performed treatment using PDL in elderly patients with vascular malformations in whom no significant effects had been obtained by previous laser treatment and examined tissue changes after laser irradiation, as a first step towards more effective laser treatment. It should be noted that we used the conventional PDL in the present study, but the long-pulsed dye laser (LPDL) has also become available, where the pulse width in the former is in the microsecond domain, and in the millisecond domain in the latter. It has been suggested that the LPDL offers greater efficacy in treatment of such lesions in children, but no such study has been performed in elderly patients, although it is possible that the longer pulse width might have greater efficacy in vascular malformations in the elderly. The much longer pulse width for the LPDL combined with the high absorption in melanin at the dye laser waveband necessitates fairly aggressive skin cooling to protect the epidermis from damage in LPDL treatment. In the case of the elderly patient, in whom the presence of cutaneous melanogenic dyschromia is prevalent, the LPDL would…
need to be applied with extreme caution. However, the potential for LPDL treatment to have a better result for laser-resistant lesions in the elderly merits exploration.

Before laser irradiation, an increase in the vasculature was observed with both light and electron microscopy, and an increase in fibroblasts was also observed in the dermis with light microscopy. However, electron microscopy showed that the structure of the blood vessels and endothelial cells was normal. These findings were different from those in children in whom the cellular characteristics of the vasculature differ from adults. One week after laser irradiation, there was extensive interstitial edema around blood vessels, but the structure of the blood vessels and capillary endothelial cells was normal and these vascular characteristics were maintained 3 months after irradiation.

We have reported that interstitial and endothelial cell edema was induced by PDL irradiation even at a fluence of 3 J/cm², which was much lower than that used in this study (7 J/cm²). However, the high fluence of 7 J/cm² induced interstitial edema but not endothelial cell edema in this study. The same device was used, and the small edematous changes in the vasculature regardless of the high fluence were considered to be specific to the elderly. This was suggested to be caused by decreased blood flow in the blood vessels of the elderly, hence a smaller amount of the major chromophore for the PDL, namely hemoglobin, was physically present in the blood vessels.

We have also reported that mast cells and lymphocytes were observed immediately and 1 week after PDL irradiation at 3 J/cm², respectively, and in these previous studies, there was also marked neutrophil and monocyte infiltration compared to this study. The weak inflammatory cell infiltration in this study was considered to be correlated with a decreased immune response in the elderly.

As for other interstitial changes, elastin-free elastic fibers and spiral collagen were observed before irradiation, supporting characteristic skin changes in the elderly. One week after irradiation, disturbed arrangement of collagen fibrils and a decrease in spiral collagen were observed, and 3 months after irradiation, findings similar to those before irradiation were obtained. The changes observed 1 week after irradiation were considered to be consistent with changes accompanied by interstitial edema.

Campolmi et al. reported that dividing cells and mast cells increase at first and then vascular cavities are formed in vascular malformations with abundant cellular components in children, and that fibrosis of the cellular components and dilated vasculature are typical tissue images. Considering that mast cells play a large role in dermal vascularity, mast cell infiltration in the skin of the elderly, regardless of small changes in the dermis, suggested that mast cells and their degranulation may play some role in maintaining the vasculature.

From the above, it was assumed that PDL treatment is less effective in the elderly than in the young because 1) response to PDL is weak due to the small number of red blood cells in blood vessels in the elderly; 2) denaturation of the dermal collagen structure plays some role in the maintenance of the vasculature in the interstitium with edema; and 3) release of some cytokines from mast and other cells due to inflammatory cell infiltration promotes the reconstruction of the vasculature. Vascular malformations in adults are known to be less responsive to various laser treatments, and it is recommended to start laser treatment during childhood. On the other hand, as described above, it was also reported that cellular type vasculature is poorly responsive to laser treatment.

The PDL device used in this study is considered to be the laser device of first choice for treatment of vascular indication, and diverse clinical applications have also been reported in a variety of lesions such as verruca vulgaris, molluscum contagiosum and acne and, as described above, its use for rejuvenation. This indicates that the PDL is assumed to have mechanisms of action that not only simply target hemoglobin but also induce other effects including those associated with a photothermal reaction, and perhaps even an athemal photobiocactivative reaction in the peripheral tissues as explained by Ohshiro as simultaneous low level light therapy. Treatment methods should be based on our findings to enable more effective results in the future for patients resistant to laser treatment.

Conclusions

Our results suggested that the lower efficacy of PDL treatment in the elderly was possibly due to the following factors:

- The amount of red blood cells in our subjects’ blood vessels, a major chromophore for the PDL, was markedly low, negatively affecting laser energy absorption.
- Age-related denaturation of the dermal matrix collagen may play some role in maintaining the vasculature in the interstitium with edema.
- Inflammatory cell infiltration possible leads to the
cellular release of some cytokines which favor reconstruction of the vasculature. Treatment methods must be investigated, including the indication of long-pulsed dye laser treatment, based on various histogeneses for the successful PDL treatment of vascular lesions in PDL-resistant patients.

References

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