

# Histological Responses of Cutaneous Vascular Lesions Following Photodynamic Therapy with Talaporfin Sodium: A Chicken Comb Model

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## Abstract

**Background:** Mono-L-aspartyl chlorin e6 (Talaporfin sodium) is a novel photosensitizer, and is currently being used in photodynamic therapy for various malignant tumors in combination with irradiation with a 664 nm laser. An interesting characteristic of Talaporfin sodium is that the skin photosensitivity after injection of this agent disappears faster than any other existing photosensitizers. This study examined the vascular events that occurred postirradiation in the chicken comb as a capillary malformation model after photosensitization with Talaporfin sodium.

**Materials and Methods:** A single intravenous bolus injections of Talaporfin sodium was administered to the chickens, and a 1 cm diameter area of the comb of each animal was irradiated with a 664 nm visible red laser. The gross changes in the chicken combs were recorded for 7-14 days after photodynamic therapy. For the histological examination, HE, PTAH and Azan stained sections were analyzed.

**Results:** All treated chicken combs had blanched after photodynamic therapy. Microscopy demonstrated an absence of erythrocytes and the vessel lumina were obliterated, leaving the normal overlying epidermis completely intact. Concomitantly with selective destruction of the capillaries in the target area, moderate invasion of inflammatory cells and a slight increase in the stroma were observed.

**Conclusions:** In the chicken comb model, photodynamic therapy with Talaporfin sodium effectively achieved selective destruction of the microvasculature while leaving the epidermis intact. Our results strongly suggest that photodynamic therapy with Talaporfin sodium could be a feasible method to treat dermal hypervascular lesions. (Keio J Med 58 (3) : 176–184, September 2009)

**Keywords:** photodynamic therapy, Mono-L-aspartyl chlorin e6, Talaporfin sodium, port-wine stains, vascular occlusion

## Introduction

Capillary malformations, the so-called port-wine stains (PWS), are a congenital vasculopathy consisting of an abnormal network of capillaries in the upper dermis with an overlying normal epidermis. PWS has no tendency to resolve spontaneously. The flashlamp-pumped pulsed

dye laser (PDL) at a wavelength of 585 nm and a pulse duration of 0.45 ms has been used for the treatment of PWS since the 1980s. This treatment is based on a photothermal reaction based on the principle of selective photothermolysis, first proposed by Anderson and Parrish.<sup>1</sup> Since 2000, a long pulse-duration PDL has been developed. This machine emits laser energy at a wave-

length of 585/595 nm and has pulse durations from 0.45 to 40 ms, enabling the treatment of larger diameter vessels and those more deeply located in the skin. Currently the PDL is the ‘gold standard’ for treatment of PWS worldwide.<sup>2–6</sup> However it is difficult to achieve successful results in deeper and thick lesions, and larger diameter vessels, with multiple treatment sessions being required to obtain good results.

On the contrary, photodynamic therapy (PDT) is an evolving selective cancer treatment which has been reported as being highly successful for treatment of malignant neoplasms since the 1970s.<sup>7–9</sup> PDT has a two step process. The photosensitizer is injected and allowed to circulate throughout the body, and is selectively taken up by the cells in highly mitotic tissues including tumors. The target tissue is then athermally irradiated with light at a wavelength specific to the photosensitizer. The athermal photoactivation of the photosensitizer induces an oxygen-dependent process that results in the generation of highly cytotoxic reactive oxygen species (ROS) including singlet oxygen. The release of these reactive molecules results in damage to both the tumor cells containing the photosensitizer and their microenvironment. There are several photosensitizers which have presently passed or are under clinical investigation. Hematoporphyrin derivatives (HpDs) such as Photofrin® (Porfimer sodium) have been approved for use against gastrointestinal cancer and early lung cancer.<sup>10–12</sup>

From the early 1990s, in addition to the use of PDT for tumors, PDT with HpDs (HpDs-PDT) activated with red or green laser (510–630 nm) has been studied for the treatment of cutaneous vascular lesions.<sup>13–15</sup> PDT for PWS is based on a photochemical reaction, and not a thermal reaction. HpDs-PDT has not been used worldwide because of the prolonged photosensitivity of the skin of patients who have been treated with HpDs. Patients must avoid direct exposure to sunlight or even strong artificial light in the longer visible wavebands for 3 to 4 weeks after PDT.

Mono-L-aspartyl chlorin e6 (Talaporfin sodium, Meiji-seika Kaisha Ltd., Tokyo, Japan) is a novel photosensitizer, and is currently being used in PDT for various malignant tumors, activated with a 664 nm laser.

The objective of the present study was to ascertain the feasibility of PDT with Talaporfin (Talaporfin-PDT) in the treatment of PWS. The main focus of the study concerned the vascular events that occurred postirradiation in the chicken comb as a capillary malformation model after sensitization with Talaporfin sodium.

## Materials and Methods

### *Animal Model*

The chicken comb was chosen as the model, because its histoanatomy is analogous to that found in hypervas-

cular dermal lesions. Adult male chickens weighing between 1.8–2.1 kg were used. After injection of the photosensitizer, the rest of the experiment, including housing of the animals, was conducted in the dark to avoid photoactivation of the photosensitizer other than by the laser.

### *Photosensitizer and Laser System*

Talaporfin sodium is a hydrophilic compound manufactured by coupling aspartic acid and chlorin, which is extracted and refined from plant chlorophyll. The drug was reconstituted as a 5.0 mg/ml solution in physiological saline immediately before administration in order to avoid degradation by light due to photochemical activity.

Talaporfin sodium is maximally activated at 410 nm with a significantly lesser peak at 664 nm. The 664 nm laser has been used in PDT. As a laser light delivery system in the present study, an aluminum gallium indium phosphorus (GaAlInP) diode laser was used, (Panalas 6450, Panasonic Ltd., Osaka, Japan), emitting at 664 nm. The laser wavelength was set at 664 nm and the output was variable in the range of 50–500 mW at the fiber tip in continuous wave mode.

### *Experimental Procedures*

Adult cockerels received a single intravenous bolus injection of Talaporfin sodium 2.5 or 5.0 mg/kg. A 1 cm diameter area of the comb of each animal was irradiated with 664 nm laser light. The total light dose was 25 or 50 J/cm<sup>2</sup> at a power density of 127 mW/cm<sup>2</sup>.

### *Study 1*

The first part of the study was to determine the optimal time for laser irradiation after injection of the photosensitizer and to determine the optimal laser dosimetry to destroy the comb microvasculature, as the metabolism of Talaporfin sodium in the chicken model had not been reported. Four cockerels were given intravenous injections of Talaporfin sodium at the above-mentioned doses. Immediately and at 30 minutes after injection, the combs were irradiated with laser light (**Fig. 1**) at a total dose of 25 or 50 J/cm<sup>2</sup>, with a power density of 127 mW/cm<sup>2</sup>. There were another two controls, one which received photosensitizer without laser irradiation, and the other which received laser irradiation without the photosensitizer. The irradiated areas were recorded daily with macrophotography for 2 weeks. All chickens were histologically examined at 14 days after PDT.

### *Study 2*

The objective of the second part of this study was to analyze how Talaporfin-PDT injured the microvasculature and affected the connective tissues surrounding the



**Fig. 1** Experimental schema.  
1: Irradiation area immediately after injection.  
2: Irradiation area 30 min after injection.



**Fig. 2** Chicken comb (subject no. 4) 14 days after PDT.  
1: The area immediately after injection was clearly blanched, equaling the irradiated area.  
2: The area 30 min after injection showed no remarkable findings.

**Table 1** Results of macroscopic findings in Study 1

Subject No.	Dose administered	Irradiation immediately after injection	Irradiation 30min after injection
1	5 mg/kg	○:50 J/cm <sup>2</sup>	×:50 J/cm <sup>2</sup>
2	5 mg/kg	○:25 J/cm <sup>2</sup>	×:25 J/cm <sup>2</sup>
3	2.5 mg/kg	○:50 J/cm <sup>2</sup>	×:50 J/cm <sup>2</sup>
4	2.5 mg/kg	○:25 J/cm <sup>2</sup>	×:25 J/cm <sup>2</sup>
5	5 mg/kg	×:0 J/cm <sup>2</sup>	×:0 J/cm <sup>2</sup>
6	0 mg/kg	×:25 J/cm <sup>2</sup>	×:50 J/cm <sup>2</sup>

Blanching was observed only in the area irradiated immediately after injection.

○:Blanching was demonstrated.

×:No change was observed.

destroyed vessels.

Eight cockerels received a single intravenous bolus injection of Talaporfin sodium 2.5 mg/kg, and immediately after injection a 1 cm diameter area of the comb was irradiated with 664 nm laser light at the same parameters. Another two control cockerels were used as in Study 1. The gross changes in the chicken combs were recorded for 7 days after PDT. Tissue specimens were routinely processed for histological evaluation. Hematoxylin-eosin (HE), phosphotungstic acid hematoxylin (PTAH), and Azan stained sections were analyzed at 3 and 7 days after PDT to observe the histological changes associated with the blanching.

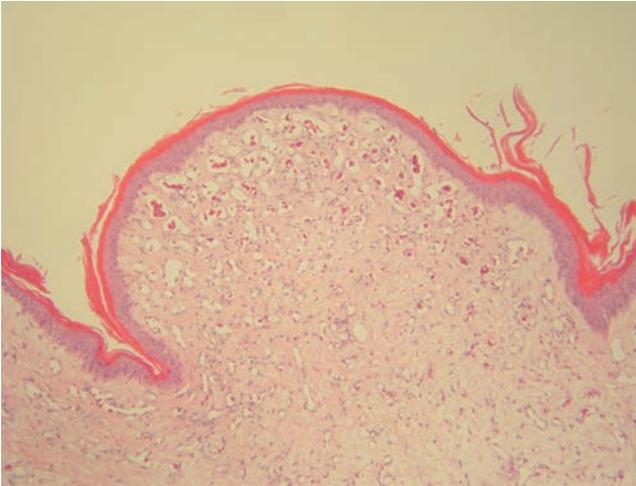
## Results

### *Optimal timing for irradiation*

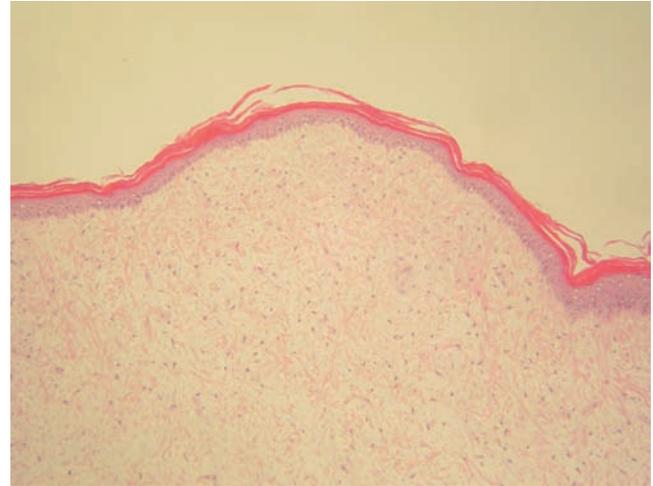
**Table 1** shows the results of the macroscopic findings.

From Study 1, blanching was observed only in the area irradiated immediately after injection (**Fig. 2**). **Figure 3** shows the normal microvasculature of the chicken comb. The microvasculature under the epidermis consists of multiple capillaries lined with flat, elongated endothelial cells and filled with erythrocytes. **Figure 4** shows the histological changes in the area irradiated immediately after injection at 14 days after PDT. In the HE stained sections the normal microvasculature was destroyed but the overlying epidermis remained intact. However, in the area 30 min after injection, there were no significantly different histological findings, as compared with the normal vasculature of the chicken comb. Furthermore the control animals, which received photosensitizer without irradiation or irradiation without photosensitizer, showed the normal comb vasculature.

Following both the Talaporfin injections of 2.5 mg/kg or 5.0 mg/kg, selective destruction of the microvasculature was observed, but no difference was seen in the de-



**Fig. 3** Normal histological findings of the chicken comb. The microvasculature under the epidermis consists of multiple capillaries lined with flat, elongated endothelial cells and filled with erythrocytes.



**Fig. 4** Histological findings of an area irradiated immediately after injection 14 days after PDT (subject No 4) (HE staining,  $\times 100$ ).

Normal microvasculature cannot be seen under a normal intact epidermis.

**Table 2** Results of macroscopic findings in Study 2

Subject No.	Dose administered	Total irradiation dose 25J/cm <sup>2</sup>	Total irradiation dose 50J/cm <sup>2</sup>
1	2.5 mg/kg	○	○
2	2.5 mg/kg	○	○
3	2.5 mg/kg	○	○
4	2.5 mg/kg	○	○
5	2.5 mg/kg	○	⊙
6	2.5 mg/kg	○	○
7	2.5 mg/kg	○	⊙
8	2.5 mg/kg	○	○
9	2.5 mg/kg	$\times$ :0 J/cm <sup>2</sup>	$\times$ :0 J/cm <sup>2</sup>
10	0 mg/kg	$\times$ :25 J/cm <sup>2</sup>	$\times$ :50 J/cm <sup>2</sup>

All irradiated areas were blanched 7 days after PDT. Superficial epidermal necrosis was observed on the No.5 and No.7 chicken combs which had been irradiated with 50 J/cm<sup>2</sup>.

○: Blanching was demonstrated.

⊙: Blanching was achieved, but slight Talaporfin-PDT-mediated epithelial injury was observed.

$\times$ : No change was observed.

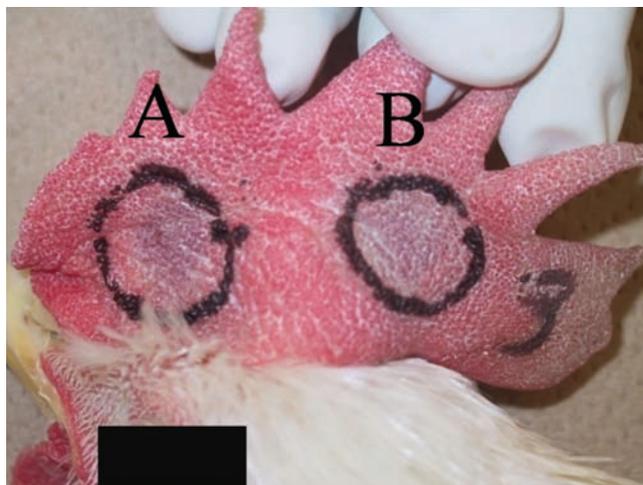
gree of destruction between the two doses of both the drug and the irradiation. Our study results suggest that at least a single bolus injection of Talaporfin 2.5 mg/kg activated with 644 nm laser at 25 J/cm<sup>2</sup> is required to achieve selective destruction of the microvasculature.

In this chicken combs model, it is recognized that the damaged blood vessels will be regenerated and revascularization will occur due to the nature of the comb, so it may turn out to be impossible to achieve permanent blanching. From the macroscopic and histological findings in Study 1, the revascularization was not confirmed until day 14.

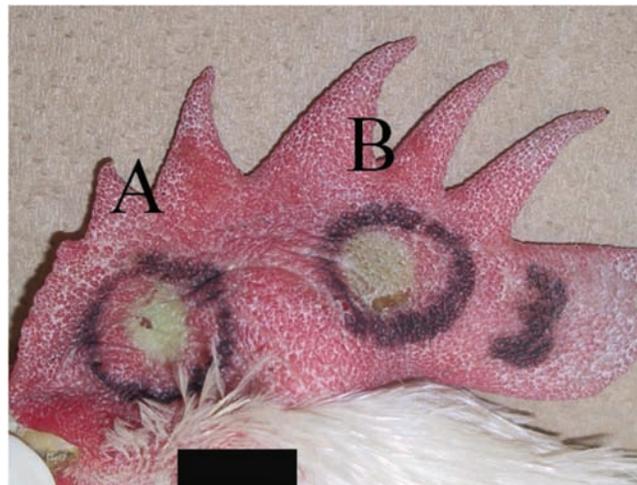
The 2 main points shown by Study 1 were that the selective destruction of the microvasculature in the superficial dermis could be achieved with Talaporfin-PDT under a normal intact epidermis, and that irradiation should be started immediately after administration.

#### Gross observations

**Table 2** shows the results of Study 2. Talaporfin-PDT could achieve blanching of the chicken combs. Immediately after PDT, all area appeared darkened following laser irradiation at both 25 J/cm<sup>2</sup> and 50 J/cm<sup>2</sup> (**Fig. 5**).



**Fig. 5** Macroscopic changes immediately after PDT (subject No 3). The entire area appears darkened. A: 644 nm laser, 25 J/cm<sup>2</sup>, B: 644 nm laser, 50 J/cm<sup>2</sup>.



**Fig. 6** Macroscopic changes 7 days after PDT (subject No 3). Blanching is seen in all areas. A: 644 nm laser, 25 J/cm<sup>2</sup>, B: 644 nm laser, 50 J/cm<sup>2</sup>.

The darkened phase continued for 3 or 4 days, pronounced blanching appeared in all irradiated areas until 7 days after PDT (**Fig. 6**).

Macroscopically there were no differences in the degree of blanching at both light doses, but crust formation due to partial epidermal necrosis appeared in some of the 50 J/cm<sup>2</sup> group.

#### *Histopathological evaluation*

**Figure 7** shows the histological findings 3 days after PDT. In the darkened area, HE and PTAH stained sections showed that the capillaries were filled with thrombus and degeneration of the endothelial cells, whereas the overlying epidermis was intact. Moderate lymphocytic infiltration was moreover observed around the degenerated vessels (**Fig. 7a, b**). Azan staining showed slight stromal increase, especially in collagen fibers around the degenerated vessels (**Fig. 7c, d**).

**Figure 8** shows the histological findings 7 days after PDT. HE stained sections showed that the capillaries were destroyed and had disappeared (**Fig. 8a**). The lumen of the larger caliber capillaries was occluded with fibrin (**Fig. 8b**). Normal endothelial cells were not seen. Azan stained sections showed that the stroma under the epithelium had increased in the upper dermis, collagen fibers had increased in number and were arranged irregularly (**Fig. 8c, d**). In some cases, partial epidermal necrosis was observed in the areas with stronger irradiation.

In the histological sections, normal microvasculature consisted of microvessels smaller than 120  $\mu\text{m}$  in the chicken combs. 3 days after PDT, the thrombus of the capillaries were confirmed to the depth of more than 1600  $\mu\text{m}$  from the dermoepidermal junction. 7 days after PDT, the microvasculature was destroyed and had disap-

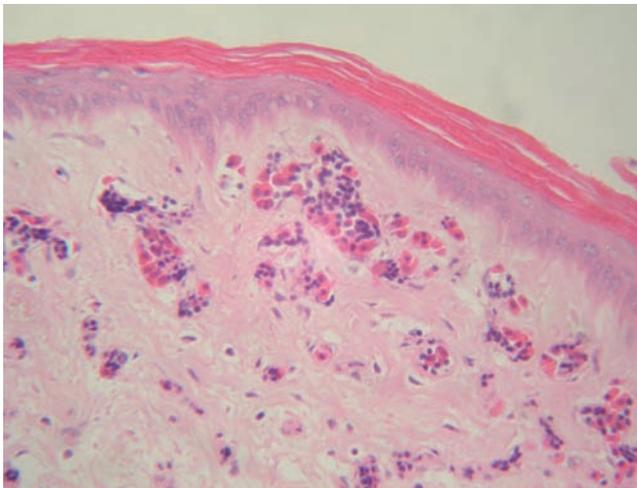
peared in the irradiated area.

#### **Discussion**

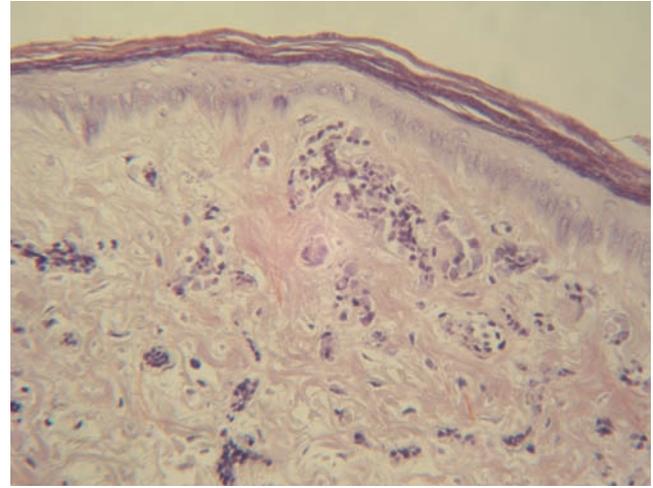
Talaporfin sodium is a second generation photosensitizer that has been shown to be effective in preclinical and clinical studies including the treatment of various malignant tumors and age-related macular degeneration.<sup>16–20</sup> Talaporfin sodium has a strong absorption peak at 664 nm and produces high yields of triplet oxygen when excited. This absorption peak at the longer visible red wavelength provides deeper penetration of light into the target tissue than at shorter wavelengths such as green or yellow light which have competing chromophores, namely melanin and hemoglobin. A significant advantage of PDT with Talaporfin sodium is the reduced duration of skin photosensitivity as compared with other photosensitizers such as Photofrin®, one of the well known HpDs. Kessel demonstrated that the kinetics of Talaporfin elimination from plasma is consistent with a half life ( $T_{1/2\beta}$ ) of approximately 134 hours, which is much shorter than the approximately 250 hours of Photofrin®. Talaporfin is eliminated almost twice as rapidly as Photofrin®.<sup>21</sup> We therefore considered the application of Talaporfin-PDT for cutaneous vascular disorders.

From our study, the results of Talaporfin-PDT for PWS using the chicken comb model demonstrated that selective destruction of the superficial dermal microvasculature could be achieved with PDT, leaving the overlying epidermis intact. This demonstrates the potential efficacy of Talaporfin-PDT as a novel modality for the treatment of PWS.

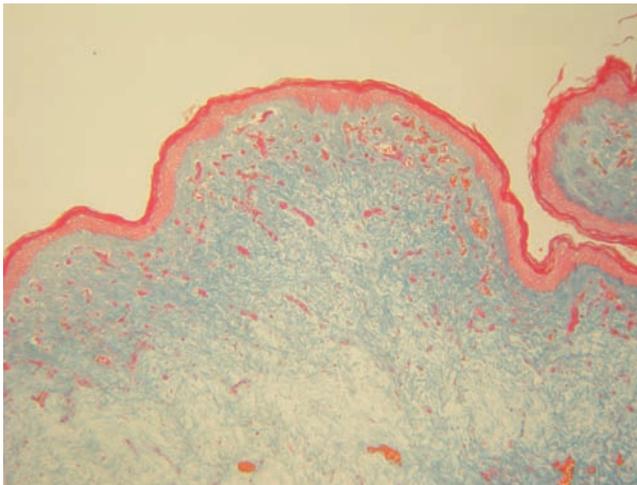
In Study 1, the optimum timing of irradiation after photosensitization was determined. Blanching was observed only in the area irradiated immediately after in-



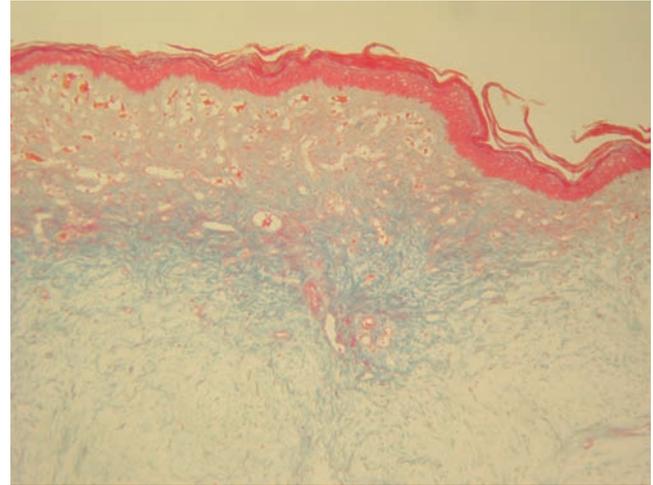
a



b



c



d

**Fig. 7** Histological findings at 3 days after Talaporfin-PDT (subject No 4).

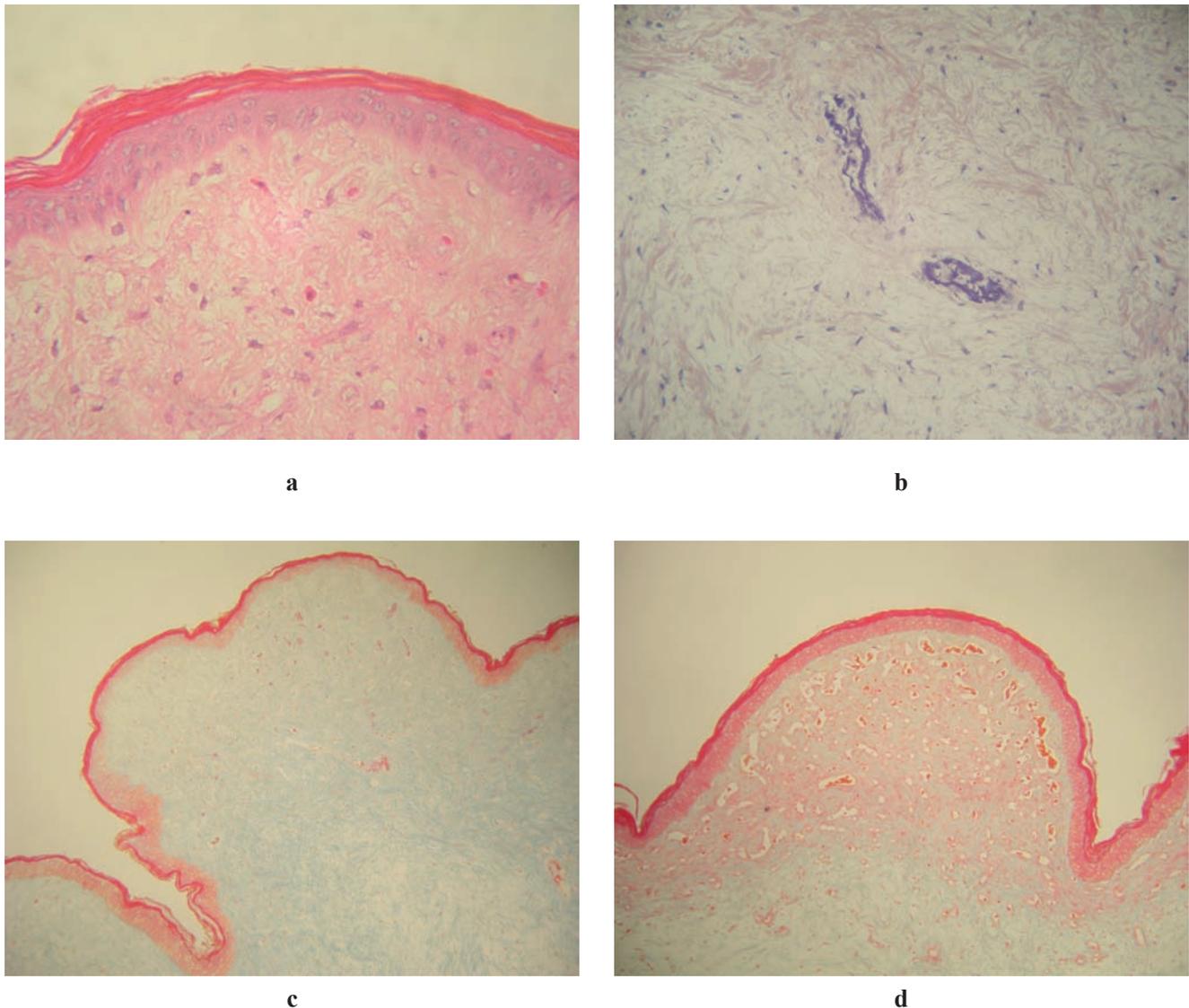
A darkened area of the comb is seen following Talaporfin injection 2.5 mg/kg and irradiation with the 644 nm laser at 25 J/cm<sup>2</sup>.

- (a): Capillaries are filled with erythrocytes and the endothelial cells have degenerated, whereas the overlying epidermis is intact (HE staining  $\times 400$ ).  
 (b): Capillary lumina are blocked with thrombus (PTAH staining  $\times 400$ ).  
 (c): Under the intact epidermis, a mild stromal increase, especially of collagen fibers around degenerated vessels, can be observed (AZAN staining  $\times 100$ ).  
 (d): Chicken comb of subject No 9 as control (AZAN staining  $\times 100$ ).

jection. The pharmacokinetics of Talaporfin sodium in the chicken model are not clear, but it is considered that the concentration of the drug in the vascular component is associated with the selective destruction of the microvasculature. A longer time period after intravenous injection would allow diffusion of the photosensitizer from the targeted microvessels into the interstitial components. It is suggested that a minimum interval is necessary for adequate concentration of the photosensitizer to be distributed in the microvasculature. PDT under condi-

tions of high plasma photosensitizer levels in the targeted microvessels implies a photodynamic-mediated vascular mechanism of selective destruction.

After Talaporfin-PDT, the irradiated areas changed from being dark-colored to blanched. During the darkening phase, the capillaries were filled with thrombus and the endothelial cells had degenerated, but under a normal and intact epidermis. In the blanching phase, the capillaries were destroyed, and the lumen of the larger caliber capillaries was occluded with fibrin. Normal endothelial



**Fig. 8** Histological findings at 7 days after Talaporfin-PDT (subject No 3).

A blanched area of the comb can be seen following Talaporfin injection 2.5 mg/kg and irradiation with the 644 nm laser at 25 J/cm<sup>2</sup>.

(a): The capillaries have been destroyed and have disappeared: normal endothelial cells cannot be observed (HE staining  $\times 400$ ).

(b): The lumen of the larger caliber capillaries remains occluded with fibrin (PTAH staining  $\times 400$ ).

(c): The stroma under the epithelium has increased in the upper dermis and an irregular linear arrangement of the increased collagen fibers can be observed (Azan staining  $\times 100$ ).

(d): Chicken comb of subject No 9 as control (AZAN staining  $\times 100$ ).

cells were not seen in the superficial part of the upper dermis. Several recent reports have concluded that tumor destruction after PDT using Talaporfin sodium is dependent on vascular damage and blood flow stasis.<sup>22-24</sup> McMahon *et al.* reported that blood flow stasis was the result of platelet aggregation and mechanical obstruction of the flow rather than vessel constriction after Talaporfin-PDT in a tumor model. Our study suggested the same mechanism occurred in a cutaneous vascular model. Limited local ischemia caused by erythrocyte congestion

and thrombi formation after Talaporfin-PDT leads to the degeneration of endothelial cells, and the selective destruction of microvasculature can be achieved.

As a result of Talaporfin-PDT, the stroma around the microvasculature increased slightly in the upper dermis. Previous reports have not referred to the microenvironment around the target microvasculature after PDT, but the results of the present study suggest that Talaporfin-PDT for PWS could possibly allow selective destruction of the microvasculature, but with minimal injury to the

tissues around the target vessels.

In Talaporfin-PDT for PWS in our animal model, the adequate regimen to achieve the destruction of the microvasculature was a single intravenous bolus of Talaporfin 2.5 mg/kg activated with 25 J/cm<sup>2</sup> of 644 nm laser light. When 25 J/cm<sup>2</sup> was compared with 50 J/cm<sup>2</sup>, partial epidermal necrosis was observed in some of the 50 J/cm<sup>2</sup> group, suggesting that this dose was too strong. Although our results indicate that the degree of photodynamic reaction in both epidermis and dermis is dependent on the PDT dose, more research is required to precisely define conditions such as the dosimetry for both the photosensitizer and light.

PDT is different from a photothermal treatment such as PDL. Using an athermal photochemical reaction, Talaporfin-PDT was able to selectively destroy the microvasculature in the upper dermis in our chicken comb model. According to the measurement of the histological sections, Talaporfin-PDT could destroy the microvasculature at a depth of more than 1600 µm from the dermo-epidermal junction and consisted of microvessels with a diameter of 20-120 µm. Although there are histological differences between human skin and chicken comb, our findings suggest that Talaporfin-PDT has the possibility to treat the deeper part of dermis. The greater penetration caused by the longer visible red wavelength (644 nm > 585/595 nm) would make it ideal for the treatment of capillary malformations which are more deeply located and consist of microvessels with various diameters.

Furthermore, a clinical trial of Talaporfin-PDT for cutaneous vascular malformation in humans is currently underway having received permission from the Institutional Review Board of Keio University School of Medicine in 2007.

### Conclusions

In conclusion, Talaporfin-PDT effectively achieved the selective destruction of the microvasculature under an intact and normal epidermis in the chicken comb model. It is thought that the PDT mechanism resulted in vascular occlusion by thrombosis caused by a photochemical reaction, but the interaction between vascular endothelial cells and the photosensitizer has yet to be elucidated. Further study is warranted to determine the optimum conditions, namely the optimum dosimetry of both the photosensitizer and laser irradiation. The results from the present study show that photodynamic therapy with Talaporfin sodium may be a feasible method to treat benign hypervascular dermal lesions in humans.

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