

## Effects of photodynamic therapy using talaporfin sodium (Laserphyrin<sup>®</sup>) on wound healing in an animal model

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**Background:** Photodynamic therapy (PDT) is an effective laser treatment for locally advanced carcinoma and is promising as neoadjuvant chemotherapy before surgery. The aim of this study was to clarify the adverse effects of PDT using a photosensitizer, talaporfin sodium (Laserphyrin<sup>®</sup>), for wound healing.

**Methodology:** For PDT, a laser light with a wavelength of 660 nm and a frequency of 10 Hz with a total energy fluency of 60 J/cm<sup>2</sup> was used. Macroscopic and histological findings of wound healing after PDT were examined in vivo (4-week-old male BALB/c mice).

**Results:** In Model 1, in which skin was cut at 0, 3, 7 days after PDT (n=3, each), wounds were similarly healed 7 days after cutting in all groups, and regenerating epithelium and the number of fibroblasts on histological findings were not different. In Model 2, in which skin defects were created before or after PDT, the size of the defects was larger at day 7 in the groups with skin defects before or after PDT in comparison with groups with no PDT.

However, macroscopic wound healing at day 14 was complete in all groups and there were no significant differences among the groups by this point. Histological findings of skin defects at day 14 showed no significant difference in terms of regenerating epithelium and number of fibroblasts in each group with or without PDT.

**Conclusions:** PDT did not influence wound healing and can be safely applied before surgical therapy.

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**Key words:** Bile duct carcinoma, Photodynamic therapy, Talaporfin sodium, Wound healing, Adverse effect

### Introduction

Photodynamic therapy (PDT) is an anti-cancer-specific treatment based on the use of light to activate photosensitizers and induce cytotoxicity in targeted cancer cells, and PDT has been widely applied in various cancer treatments.<sup>1)</sup> Powerful anti-tumor immunological responses are remarkably induced by PDT.<sup>2)</sup> PDT also provides longer survival in non-resectable tumors<sup>3)</sup>. We have reported the benefits of PDT treatment in patients with bile duct carcinomas undergoing

chemotherapy or adjuvant chemotherapy after surgery for local control.<sup>4)</sup> Thus, PDT may be a promising treatment modality when added to conventional anti-cancer chemotherapy and brachytherapy<sup>5)</sup>.

We recently reported a new and effective photosensitizer, mono-L-aspartyl chlorin e6 (talaporfin sodium; NPe6, Laserphyrin<sup>®</sup>), which has been clinically used for treatment in malignant tumors such as a bronchial or biliary carcinomas.<sup>4),6)</sup> The 664 nm wavelength of semiconductor laser light activates talaporfin sodium, penetrating the deep tis-

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sue more than 10mm.<sup>6)</sup> PDT with this photosensitizer showed powerful effectiveness in anti-cancer treatment, including high rates of tumor necrosis and apoptosis, lower proliferative activity of cancer cells and higher anti-angiogenic activity.<sup>7)</sup> Combination therapy with PDT and conventional anti-cancer drugs resulted in more aggressive cytotoxic effects, and therefore this new therapy can be clinically applied as chemotherapy in non-resectable tumors, adjuvant chemotherapy after surgery and neoadjuvant chemotherapy before surgery in advanced carcinomas to prolong survival periods. In considering this therapy for neoadjuvant chemotherapy in solid tumors, the effect of PDT on wound healing in irradiated normal tissue is a major concern because inflammation may delay healing<sup>8)</sup>. It is necessary to clarify whether PDT using talaporfin affects wound healing.

In the present study, we examined wound healing in skin resected both before and after PDT in an *in vivo* mice model to address these concerns.

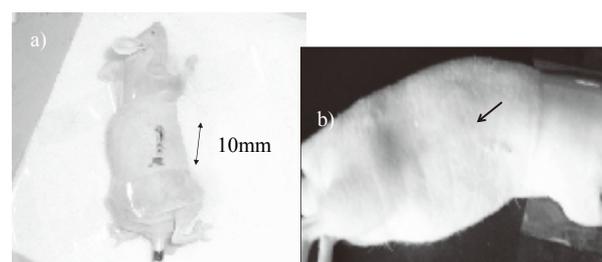
## Method

### PDT protocol

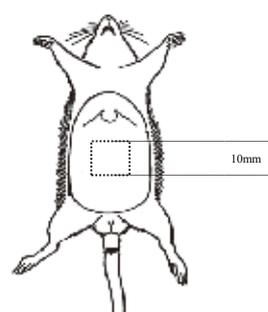
A power meter (30 A-P Ophir Optics, Jerusalem, Israel) was used to measure light intensity. Talaporfin sodium was injected intraperitoneally into BALB/cANcrj nu/nu mice. The time interval between photosensitizer injection and light exposure for talaporfin sodium was 2 hours. Each mouse received a total energy fluency of 10mJ/cm<sup>2</sup>/pulse (total 60 J/cm<sup>2</sup>) on the back or abdomen for 10 minutes. During laser light exposure, the animals were anesthetized with intra-peritoneally injected pentobarbital sodium (40 mg/kg body weight). PDT was directly radiated to the wound sites on the back or abdomen of the animal.

(Protocol 1) Mice underwent skin incision up to the muscular fascia in 10mm sites at 0, 3, 7 days after PDT exposure (n=3, each) (Figure 1). Neither the photosensitizer nor laser light were used in the control animals (n=3). The mice were sacrificed at 7 days after incisions were made. Incised specimens were histologically examined by conventional hematoxylin-eosin stain.

(Protocol 2) A 10mm x10mm skin defect with the muscular fascia was created in the mouse abdomen at sites that were exposed to PDT laser (n=3, each) (Figure 2). Neither the photosensitizer nor laser light were used in the control animals (n=3). The skin defect operation was performed after



**Figure 1.** *In vivo* skin incision on the nude mouse back (a) 10mm skin incision extending to the muscular layer (b) the healed wound.



**Figure 2.** *In vivo* 10x10mm skin defect extending to the muscular layer on the nude mouse abdomen.

PDT in one group (n=3), and the operation was performed prior to PDT in another group (n=3). In a group of PDT before skin defect, resection was immediately performed after a laser exposure. In a group of PDT after skin defect, the photosensitizer was immediately. In each group, the area of wound defect was measured by the median maximum and minimum length (mm) at 2, 4, 7, 9 and 14 days and the mice were sacrificed at 14 days after PDT. Incised specimens were histologically examined by the conventional hematoxylin-eosin stain.

### Statistical analysis

Data are expressed as mean  $\pm$  standard deviation (SD). Statistical significance was determined by two-way multi-repeated ANOVA, one-way factorial ANOVA and multiple comparison tests by Scheffe's test using the statistical package StatView (Abacus Concepts Inc., Berkeley, CA). Furthermore, the Mann-Whitney U-test was used for evaluation of percentages of stained cells. A *P* value less than 0.05 was considered statistically significant.

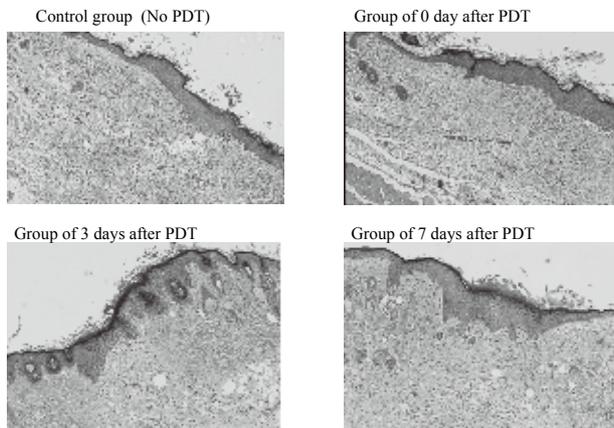
## Results

### (Protocol 1) Skin cut at 0, 3, 7 days after PDT (n=3, each)

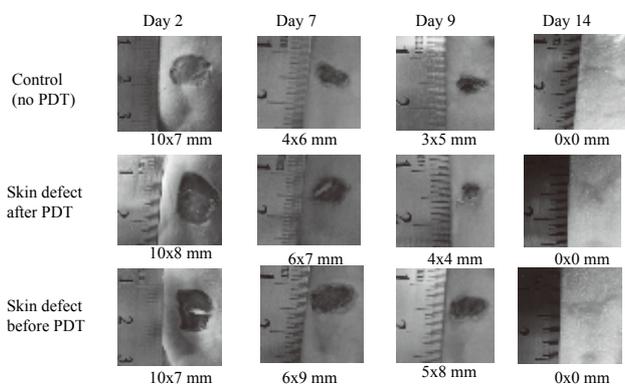
Seven days after cutting, the wounds were macroscopically healed in all groups. Figure 3 shows the histological findings of the wounds. There were no remarkably differences in regenerating epithelium or the number of fibroblasts.

### (Protocol 2) Skin defect before or after PDT

Figure 4 shows the macroscopic changes in the skin defects. Wounds were similarly healed at 14 days in all groups. However, up to day 7, the defect size was larger in groups exposed to PDT before or after skin defect in comparison with groups with no PDT exposure.

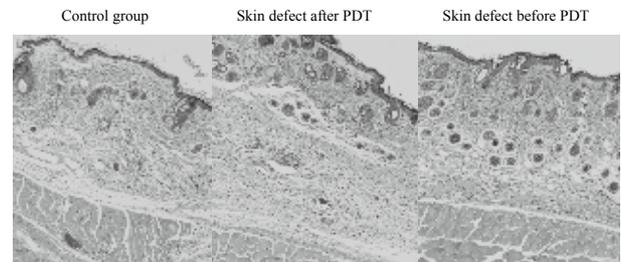


**Figure 3.** Histological findings at day 7 after skin incision. Comparison among groups with 1) no PDT (n=3), 2) incision just after PDT (n=3), 3) incision at day 3 after PDT (n=3) and 4) incision at day 7 after PDT (n=3), respectively. (x4).



**Figure 4.** Changes of 10x10mm skin defect with or without PDT. Comparison among groups with 1) no PDT (n=3), 2) skin resection after PDT (n=3) and 3) skin resection before PDT (n=3), respectively.

Figure 5 shows the histological findings of skin defect at 14 days. Compared to control (no PDT), there were no remarkable differences of regenerating epithelium or number of fibroblasts in the groups that underwent PDT before or after skin defect.



**Figure 5.** Histological findings at day 14 after skin resection. Comparison among groups with 1) no PDT (n=3), 2) resection after PDT (n=3), and 3) resection before PDT (n=3), respectively. (x4).

## Discussion

In the present study, we compared the influence of PDT using talaporfin sodium on wound healing. Previous studies showed that the main effect of PDT is apoptosis of cultured cells with DNA fragmentation or chromatin condensation.<sup>7)</sup> Increased laser or drug concentration in PDT induced cell damage in normal cells based on the MTT assay.<sup>7)</sup> Damage of normal tissues was macroscopically observed in normal bile duct tissue after PDT with porfimer sodium in our series.<sup>4)</sup> PDT induces cell damage such as apoptosis or necrosis by direct cytotoxicity of singlet oxygen or induced inflammation by immunological cells, as well as vascular shutdown effects in surrounding microvessels.<sup>9)</sup> Other investigators also reported normal tissue damage after PDT using porfimer sodium.<sup>10)</sup> Normal cell and tissue damage resulting from PDT using talaporfin sodium might be milder than that observed with PDT using porfimer sodium.<sup>11), 12)</sup> At this stage, we have applied PDT using talaporfin sodium in clinical trials and related experiments and, therefore, concerns about normal tissue damage surrounding cancerous cells seem to be relieved for future trials.<sup>13)</sup> In the future, we plan to use PDT in the neoadjuvant setting before operation in bile duct carcinomas, and therefore the influence of PDT on wound healing due to injury of normal tissue raises concerns.

Photochemical reactions depend on the level of oxygen in tumor tissues.<sup>14)</sup> PDT induces severe tumor tissue hypoxia immediately, and this is linked to the induction of photochemical reactions.<sup>15)</sup> Vascular endothelial growth factor

(VEGF) secretion is induced in cells under hypoxic conditions and other stresses.<sup>16)</sup> In our preliminary study<sup>7), 11)</sup>, expression of VEGF was used as an index of tumor tissue oxygenation and the observed overexpression of VEGF after PDT was likely due to hypoxia induced by photochemical reactions. VEGF expression within the PDT-treated lesions may influence surrounding normal tissue. In the present *in vivo* study, we examined wound healing in mice undergoing a combination of skin resection and PDT. In examination of the skin incisions in model 1, wound healing was fast and no changes among the groups were observed. Thus, this model was not adequate to examine the influences of PDT for wound healing. In the second model, wound healing was delayed up to 7 days in groups that were exposed to PDT before or after skin resection in comparison with the groups with no PDT exposure. Based on these results, PDT might delay healing, as previously reported.<sup>8), 17), 19)</sup> Accumulation of the photosensitizer might be immediately metabolized in normal cells within a couple of hours, and the laser irradiation might not strongly damage surrounding normal skin.<sup>12)</sup> Other reports also showed less damage to normal tissues by PDT using talaporfin sodium.<sup>12), 20)</sup> Under this basic theory, however, tissue injury by a photochemical reaction with an inflammation might be remained and the very small amount of photosensitizer might be retained in the normal tissue. Possibility of these influences could not be denied. VEGF might be expressed as a result of vascular shutdown effect by PDT using talaporfin sodium.<sup>21)</sup> Damage injury of surrounding microvasculatures around a skin defect is caused by the PDT effect as well. Thus, impairments of angiogenesis associated with wound healing were supposed to be delayed wound healing by day 7. In the present study, differences of the conventional histological findings between groups were not observed. It should be necessary to examine the histological findings at day 7 and additional histochemical examinations for evaluations of microvessel or elastic fiber stains.<sup>22), 23)</sup> In the present model, the interval between PDT procedures and an operation of skin defect was quite short and, therefore, it should be necessary to clarify the adequate interval between treatments to avoid influences by PDT by considering the future clinical applications. However, at day 14, wounds were completely healed in all groups and PDT did not delay healing. Thus, PDT using talaporfin sodium might not have a remarkable effect on wound healing. Likewise, the order of PDT, either before or after skin resection, did have any effect on healing. Regardless of delayed healing by day 7, the final healing was similar between groups. Definite reasons was not fully explained and, however, the inflammatory findings were observed for

one week after porfimer-sodium PDT by our previous experiences.<sup>4)</sup> After this acute phase, inflammation in the radiated parts immediately dissipated. Inflammatory changes by PDT were mild in comparison with other laser treatments. It is necessary to study the further examination the mechanism of healing during between day 7 and 14. Another limitation of this study was a method of measuring area of skin defect. More accurate are measurement of skin defect should be examined by an image analysis to evaluate the wound healing effects.

In conclusion, we examined the influence of PDT using talaporfin sodium on wound healing in normal tissues in an *in vivo* mouse model. One centimeter skin incisions and defects before or after PDT were made and wound healing was examined. In both models, complete wound healing was not significantly different in groups with or without PDT exposure. PDT using talaporfin sodium is considered safe for wound healing in normal tissues, and therefore, neoadjuvant treatment before surgery can be considered. Future studies regarding PDT for an anastomosis model in digestive organs is necessary before a clinical trial is conducted.

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