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ORIGINAL ARTICLE – CLINICAL ONCOLOGY

A photodynamic therapy combined with topical 5-aminolevulinic acid and systemic hematoporphyrin derivative is more efficient but less phototoxic for cancer

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Abstract
Purpose Although photodynamic therapy (PDT) has been shown to be effective in cancer treatment, its side effects, such as a long-lasting skin photosensitivity after the application, still cause patient's inconvenience. In this retrospective cohort study, our objective was to explore a more efficient but less phototoxic PDT for skin cancers.
Methods The PDT combined with a topical photosensitizer 5-aminolevulinic acid (ALA) and an intravenously injected light-sensitive agent hematoporphyrin derivative (HPD) was used to treat 26 patients with 41 skin cancer lesions in head and face. The findings were then compared with the results of the HPD-PDT alone and the ALA-PDT following CO_2 laser ablation on 28 and 41 skin cancer patients, respectively.
Results The complete remission rate for the combined PDT was 100 % in 2 months and 97.6 % in a 6 months to 6 years trial after the treatment compared with those of 92.9 and 95.1 % for the HPD-PDT and the ALA-PDT after a single treatment, respectively. Moreover, while the patient treated with the HPD-PDT needs to avoid strong light exposure for 4–5 weeks, the combined PDT significantly reduced the period to 10–14 days. Also, in the combined PDT, the dose of the HPD, a pro-toxic light-sensitive drug, was much lower than that in the HPD-PDT.
Conclusions The combined PDT not only shows high cure rate for skin cancers but also decreases the dose of the pro-toxic HPD and significantly shortens the photosensitive period, from which the patients are able to benefit.

Keywords Combined photodynamic therapy · Photosensitizer · 5-Aminolevulinic acid · Hematoporphyrin derivative · Photosensitive period

Introduction

Photodynamic therapy (PDT) is a treatment in which lasers or other light sources, combined with light-sensitive agents, are used to destroy tumor cells. Its mechanisms are generally related to three processes: first, after being topically or systemically applied, the photosensitizer will accumulate in the tumor in 2–3 days. When a light with a specific wavelength initiates the photosensitizer, the latter generates a toxic photochemical product—singlet oxygen ($^1O_2$), which then causes the apoptosis, necrosis, and autophagy of the tumor cells, followed by the reduction of the tumor size (Zhu et al. 2005; Agostinis et al. 2011; Mroz et al. 2011); second, the PDT is able to damage the blood vessels of the tumors, which inhibits the tumor growth; third, the PDT initiates the host's immune system to attack the tumor cells.

The PDT has been documented to effectively treat the cancers, such as oral cancer, lung cancer, and bladder carcinoma (Dougherty et al. 1998; Agostinis et al. 2011), e.g., a study shows that in a total of 114 patients with early oral cancers, a complete response rate of 85 % is observed in 12 weeks and 75 % of patients survive in 2 years after the PDT with systemically administered photosensitizer

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tetra-hydroxyphenyl-chlorin (mTHPC) (Hopper et al. 2004).

Skin cancer is a common form of the cancers. There are in general five standard treatments for it: surgery, radiation therapy, chemotherapy, biologic therapy, and the PDT. The PDT has exhibited a high response rate in the treatment of non-melanoma skin cancers and shows better advantages than other strategies for the skin cancer treatments. When superficial basal cell carcinomas are treated with the PDT, for instance, the rate of the complete response in 3 months can reach as high as 97 %, although the recurrence rate of 22 % is noted in a 5-year trial (Basset-Seguin et al. 2008). Moreover, compared with the surgery, the PDT shows no mortality, an outpatient treatment, shorter period of recovery, lower cost, better cosmetic outcome, etc. Meanwhile, the chemotherapy and the radiation treatments do not affect the PDT application, and the PDT's sensitivity would not be reduced by the chemoresistance and the radioresistance (Dougherty et al. 1998; Agostinis et al. 2011).

However, the main side effect of the systemical PDT is phototoxicity or photosensitivity, in which the intravenously injected photosensitizer makes the skin and eyes of the patients sensitive to sunlight and indoor bright light for several weeks (Capella and Capella 2003; Dolmans et al. 2003; Vrouenraets et al. 2003). When the patients' skin is directly exposed to bright lights, it may take only several minutes for the skin to become sunburned, swollen, and blistered. Therefore, the patient has to avoid the exposure to the light irradiations for a long period until the photosensitizer is metabolized in the body, which causes inconvenience to the patients.

In this retrospective cohort study, our objective is to explore a more convenient and efficient PDT for skin cancers. Thus, we analyzed the efficacy of the PDT, combined with a topical photosensitizer 5-aminolevulinic acid (ALA) and an intravenously injected light-sensitive agent hematoporphyrin derivative (HPD), in the treatment on 26 patients with 41 skin cancers lesions in head and face (sixteen with skin squamous cell carcinomas, nine of skin basal cell carcinomas, one with sweat gland carcinoma). It was then compared with the efficacy of the HPD-mediated PDT (HPD-PDT) or the PDT with the application of the ALA (ALA-PDT) following CO_2 laser vaporization on 28 or 41 skin cancer patients, respectively. We show here that the combined PDT not only exhibits a high cure rate for skin cancers but also decreases the dose of the pro-toxic HPD and shortens the photosensitive period of the patients, from which patients are able to benefit.

Table 1 Effect of the combined PDT on three histological types of skin carcinomas

Histological type	Number of tumors (cases)	Time of PDT	CR	SR	PR	NR
Basal cell carcinoma	14 (9)	1st	14	0	0	0
Squamous cell carcinoma	26 (16)	1st	25	1	0	0
Sweat gland carcinoma	1 (1)	1st	1	0	0	0
Total number	41 (26)	1st	40	1	0	0

There is 100 % CR rate in 2 months after the combined PDT, but the rate is reduced to 97.6 % because one squamous cell carcinoma recurs in 3 months after the treatment. The same CR rate is kept in a follow-up period of 6 months to 6 years

PDT photodynamic therapy, *CR* complete remission, *SR* significant remission, *PR* mild remission, *NR* non-remission

Table 2 Routine HPD-PDT for three histological types of skin cancers

Histological type	Number of tumors(cases)	Time of PDT	CR	SR	PR	NR
Basal cell carcinoma	13 (13)	1st	11	2	0	0
Squamous cell carcinoma	10 (10)	1st	10	0	0	0
Skin carcinoma in situ	5 (5)	1st	5	0	0	0
Total number	28 (28)	1st	26	2	0	0

26 of 28 skin cancer lesions are completely resected after the HPD-PDT, yet two pigmented basal cell carcinomas are found only SR in 5–6 weeks posttreatment. Therefore, the CR rate is 92.9 %. However, after the second HPD-PDT is applied on these two SR cancers, the CR rate of 100 % is noted over a 6 months to 6 years follow-up period

HPD-PDT hematoporphyrin derivative-photodynamic therapy, *CR* complete remission, *SR* significant remission, *PR* mild remission, *NR* non-remission

Table 3 ALA-PDT following CO_2 laser vaporization for seven histological types of skin carcinomas

Histological type	Number of tumors (cases)	Time of treatment	CR		SR	PR	NR
Basal cell carcinoma	14 (14)	1st	14		0	0	0
Squamous cell carcinoma	18 (14)	1st	16		2	0	0
		2nd for SR		2	0		
High-grade dysplasia in skin	5 (5)	1st	5		0	0	0
Neuroendocrine carcinoma in skin	1 (1)	1st	1		0	0	0
Sweat gland carcinoma	1 (1)	1st	0		1	0	0
		2nd for SR		1	0		
Adnexal carcinoma	1 (1)	1st	1		0	0	0
Cutaneous horn	1 (1)	1st	1		0	0	0
Total number	41 (41)		38	3	3	0	0

When 41 skin carcinomas are treated with a single ALA-PDT, the CR rate of 92.7 % is noted, but it is increased to 100 % after the another ALA-PDT on two incompletely treated squamous cell carcinomas and the surgical excision on one sweat gland carcinoma

ALA-PDT 5-aminolevulinic acid-photodynamic therapy, *CR* complete remission, *SR* significant remission, *PR*mild remission, *NR* non-remission

Materials and methods

Subjects

A total of 95 subjects with non-melanoma skin cancers (Tables 1, 2, 3), who had visited our hospital from 2004 to 2008 and were treated by the PDT, were enrolled for this retrospective cohort study. Among the patients, there were 42 men and 53 women, aged between 45 and 95 years. The duration of the diseases ranged from 3 to 36 months. The tumor diameters were approximately between 10 and 40 mm. The thickness of the tumors above the skin varied from 5 to 15 mm (average 10.6 ± 6.4 mm).

The PDT treatment was approved by our institutional review board. Each subject signed a written informed consent before the therapy.

Study sign

Group 1: combined PDT (PDT with ALA and HPD application)

Forty-one skin cancers of 26 subjects (Table 1), including 16 with skin squamous cell carcinoma, 9 of skin basal cell carcinoma, and 1 with sweat gland carcinoma, were treated by the PDT combined with the application of the topical photosensitizer ALA and systemic light-sensitive drug HPD (named as combined PDT).

During the combined PDT, the subject was intravenously injected with the photosensitizer HPD (HiPorfin, Chongqing Huading Modern Biopharmaceutics Co., Ltd, Chongqing, China) at a dose of 1.5 mg/kg body weight, which was diluted in 250 mL of normal saline; 48 h later, the HPD accumulation in the lesion of the skin cancer was evaluated by a photodynamic diagnosis (PDD), in which a laser beam with 405 nm wavelength was used to excite the photosensitizer HPD exhibiting an affinity to tumor cells. It emits a fluorescence spectrum with two peaks at 630 and 690 nm. When a two-peak fluorescence spectrum was noted on an optical multichannel analyzer (OMA, Princeton institute, Germany), 8 % methyl ALA cream (Metvix™, Oslo, Norway), made by mixing the ALA with an Unguentum M cream, was then applied on the lesion skin area including the normal skin of 5 mm away from the lesion for 4 h. Finally, the irradiation of 630 nm wavelength from a diode laser of 250 mW/cm^2 (CeramOptec, Germany) was used to treat each photo spot in the cancer lesions for 20 min. The wound healing time was 4–12 weeks. After the PDT, if the tumor incompletely responded to the treatment or recurred, the second PDT would be provided.

Since the peak level of the fluorescence spectrum can be affected by a series of factors, such as the distance between the end of the optical fiber and the lesion as well as the angle of the laser to the lesion, in this study, a relative intensity was used to analyze the photosensitizer levels in the lesion and the normal skin. An increase in the fluorescence intensity at 630 nm lasts a length of 40 nm, so the start point of the increase was chosen as an internal control. The ratio of the fluorescence intensity at 630 nm to the one at 610 nm was used to calculate the photosensitizer level in the body. The accumulation of the photosensitizer was defined by the difference of the ratios between the intensity at 630 nm and the one at 610 nm in the tumor and in the normal skin.

The PDD was also used to dynamically monitor the HPD level in the lesions of the skin cancer and in the normal skin before and after the photosensitizer administration, before and 3, 7, 14, and 28 days after the PDT, as well as during the follow-up duration from 6 to 18 months. Therefore, the PDD can guide the PDT application, determine the time

of avoiding light exposure of the patients, and detect tumor recurrence.

Group 2: HPD-PDT

Twenty-eight subjects with different types of skin cancers (Table 2) were conducted with the HPD-PDT at an HPD dose of 5 mg/kg, that is, during a period of 24–48 h after the intravenous administration of the HPD, when the HPD levels in the skin cancer lesions, monitored with the OMA spectrum analyzer, were dramatically elevated, the PDT was applied.

Group 3: ALA-PDT following CO_2 laser vaporization

The skin cancer lesions of 41 patients (Table 3) were treated by using an ultrapulse CO_2 laser (Fractional CO_2 Laser System Atl-250, Ao Tong Laser Technology Co, Ltd., Shanghai, China) in 10–20 W power to reduce the sizes of the tumors, followed by the PDT with 20 % ALA. At 4 h post-ALA, the PDT was performed.

Posttreatment care

The next treatment strategy right after the PDT application is to protect the skin wound from infection as described as follows: The wound of the skin was first washed with 3 % boric acid, then treated by a topical antibiotic mupirocin ointment, and finally applied with a recombinant human epidermal growth factor spray to help wound healing.

After the PDT, the patients were asked to avoid the exposure of the sunlight and strong indoor light for weeks until the light-sensitive drug was metabolized in their bodies, which was monitored with the PDD.

A follow-up appointment was scheduled once a week in first 2 months and then changed to one time every 2–4 weeks during the period of next 2 to 6 months after the treatment. While the follow-up durations for both the combined PDT and the HPD-PDT varied from 6 months to 6 years, the one of the ALA-PDTs ranged between 6 months and 4 years.

Clinical evaluation for the PDT application

To evaluate the efficacy of the PDT, the following criteria were used:

Complete Remission (CR) refers to the complete disappearance of the tumor, no pathologically observed tumor cell during a 1-month period after the PDT.

Significant Remission (SR) is that the tumor incompletely disappears, but its mass is reduced to more than 50 % of its original size in 1 month after the treatment.

Mild Remission (MR) means that the remission of the mass is less than 50 % of the original tumor post-PDT.

Non-remission (NR) refers to no any response in the mass.

Statistic analysis

The data were analyzed by Student's t test. Statistical significance was defined at $p < 0.05$. The data were shown as the means ± the standard errors of the means (SEM).

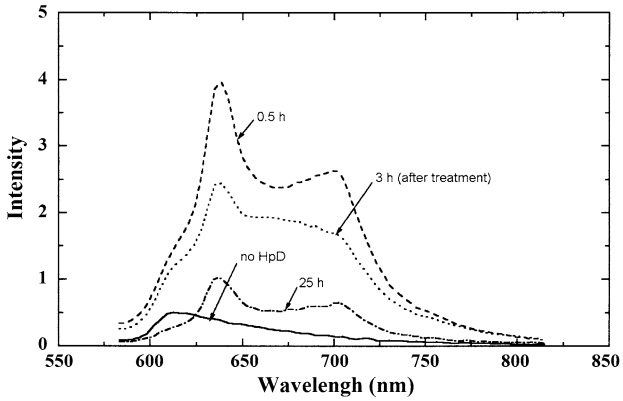

Fig. 1 Fluorescence spectra of the HPD intensity alterations in a cancer lesion after the HPD-PDT. In the lesion of the skin carcinoma, a *flat curve* is observed with no HPD. In contrast, the HPD intensity, shown by a fluorescence spectrum with two characteristic peaks at approximately 630 and 690 nm, is increased following the HPD injection, but the increased HPD intensity is decreased after the treatment

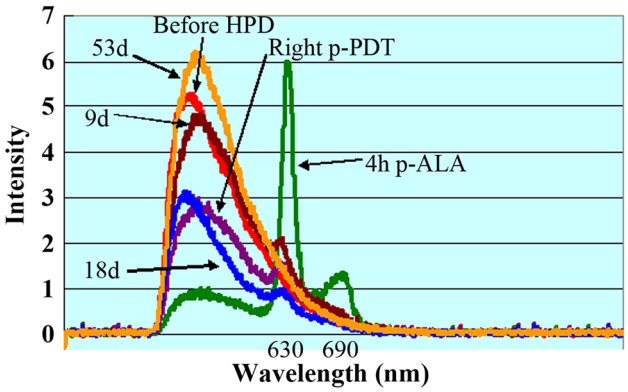

Fig. 2 Dynamic change of the fluorescence spectrum of the photosensitizers in a cancer lesion before and after the combined PDT. At 630 nm, there is a basic fluorescence intensity before the administration of the HPD (*red curve* before HPD), but the intensity is dramatically increased at 4 h post-ALA application (*green curve*, 4 h p-ALA). Right after the combined PDT, the fluorescence intensity is greatly decreased (*purple curve* right p-PDT), and it is kept around the basic level 9, 18, and 53 days post-PDT, which is represented by *brown blue*, and *orange curves*, respectively

Results

HPD level in the skin cancer lesion could be monitored by the PDD

The fluorescence spectrum, which was detected by the OMA spectrum analyzer, showed a well-nigh flat curve in the lesion of the skin carcinoma before the intravenous injection of the HPD (Fig. 1). In contrast, the HPD intensity was increased in the tumor after the HPD injection, yet the increased HPD level, the fluorescence spectrum of which exhibited two characteristic peaks at approximately 630 and 690 nm, was decreased following the HPD-PDT (Fig. 1).

In the combined PDT, a fundamental fluorescence intensity was observed at 630 nm in the lesion before the HPD administration (Fig. 2, red curve, labeled by Before PDT). In contrast, the intensity was greatly elevated after the ALA was applied for 4 h prior to laser irradiation (Fig. 2, green curve, represented with 4 h p-ALA). However, there was a dramatic decrease in the fluorescence intensity right after the combined PDT (Fig. 2, purple curve, marked by right p-PDT). The intensities were later maintained around the basic levels 9, 18, and 53 days posttreatment, which were represented by brown, blue, and orange curves, respectively (Fig. 2).

Moreover, the time course of the changes of the photosensitizer fluorescence intensity levels, represented by the relative fluorescence intensity, was also shown in a histogram before and after the combined PDT (Fig. 3). The fluorescence intensity levels in normal skin and in the lesion of a squamous cell carcinoma are marked by a yellow line and a red line, respectively (Fig. 3). After the HPD administration, the HPD levels were increased at 1 and 48 h. At 48 h postinjection, a topical light-sensitive drug ALA was also applied on the surface of the lesion. After 4 h, the photosensitizer level was greatly elevated, but it was dramatically decreased right after the irradiation and almost returned to the normal level in 4, 9, 25, and 53 days, although there was a slight increase in the intensity 18 days post-PDT (Fig. 3).

High CR rates for skin cancers were observed in the combined PDT, HPD-PDT, and ALA-PDT following CO_2 laser vaporization

The lesions of all the skin carcinomas were resected for pathological diagnosis during the PDT.

All the 41 skin carcinomas in 26 patients completely disappeared in 2 months after the combined PDT, so the CR rate of 100 % in 2 months was achieved. A representative combined PDT for a basal cell carcinoma is shown in Fig. 4. However, one squamous cell carcinoma (stage II) of a patient near the original cancer lesion recurred in 3 months posttreatment, that is, 40 of 41 skin cancer lesions were cured after the combined PDT. Therefore, the CR rate was 97.6 % in 3 months after the treatment and was kept at the same percentage during the follow-up period of 6 months to 6 years (Table 1).

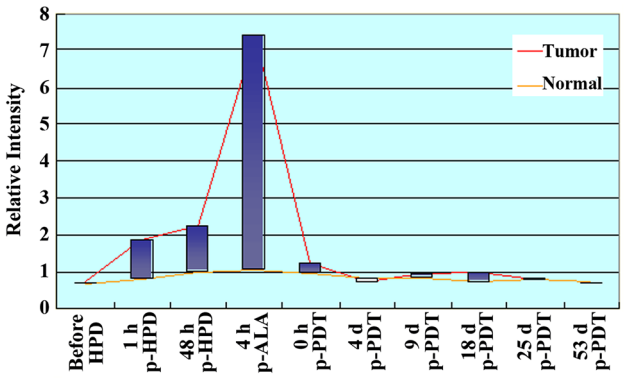

Fig. 3 Time course of the changes of the photosensitizer levels in normal skin and in the lesion of a squamous cell carcinoma before and after the combined PDT. A basic fluorescence level, represented by the relative intensity of the fluorescence, is noted in normal skin (*yellow line*) and in the lesion prior to the HPD injection. In contrast, the fluorescence level is elevated at 1 and 48 h after the HPD administration. After a topical ALA is applied on the lesion for 4 at 48 h after the HPD injection, the intensity of the fluorescence is much greater than before. However, the fluorescence level is dramatically reduced right after the PDT and near the basic level 4, 9, 25, and 53 days after the treatment, although slight increase in the intensity is noted 18 days post-PDT

In all the lesion sites, slight scars were noted after the combined PDT (Fig. 4). During the treatment, the PDD was used to monitor the photosensitizer accumulation in tumor, which can also detect tumor recurrence.

Twenty-eight skin carcinomas with three histological types, confirmed by pathological examination during the treatment, were treated by the routine HPD-PDT. The dose of the intravenously injected HPD is 5 mg/kg body weight. Among the carcinomas, while 26 tumors completely disappeared, two basal cell carcinomas (pigmented) were noted only SR in 5–6 weeks posttreatment. However, after the second HPD-PDT was applied, the two SR tumors were cured. Thus, the CR rate was 92.9 % after the first HPD-PDT and 100 % after the second treatment during a follow-up period of 6 months to 6 years (Table 2). The time of the wound healing was 4–12 weeks (6.38 ± 1.78). The patients treated with the HPD-PDT were advised to avoid strong light exposure for 4–5 weeks.

In contrast, in another group, 41 skin cancer lesions were applied with the PDT using 20 % topical ALA following the ultrapulse CO_2 laser treatment. After first ALA-PDT following CO_2 laser treatment, all tumors were cured except that three carcinomas were incompletely treated:

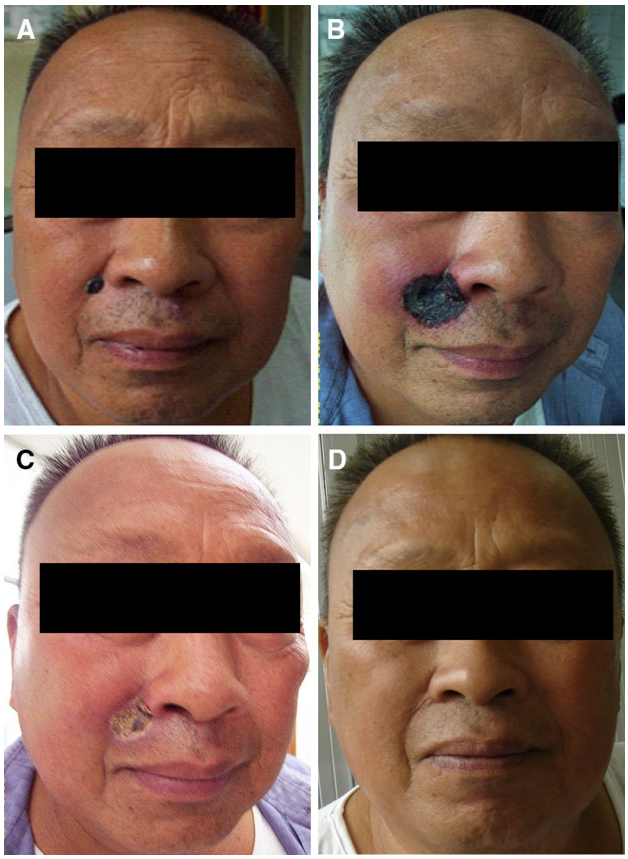

Fig. 4 Combined PDT for a basal cell carcinoma. The skin cancer lesion is located near the *right* side of the nose before the PDT (**a**). In 1 week after the combined PDT, there is edema and *thick black scab* around the wound (**b**). 3 weeks later, the *thick black scab* is fallen off, and the tumor disappears (**c**). The image **d** shows the skin in 1 year after the combined PDT. *Slight scar* is formed, but the carcinoma disappears

one squamous cell carcinoma was found not to disappear in 4 weeks, and one sweat gland carcinoma and another squamous cell carcinoma disappeared but recurred in 7 weeks and 3 months posttreatment, respectively. Therefore, the rate of the CR was 95.1 % in 2 months and 92.7 % in 3 months after the treatment. However, the CR rate of 100 % was achieved over a follow-up duration between 6 month and 4 year after the second ALA-PDT was used to treat two above squamous cell carcinomas and the surgical resection was performed for the sweat gland carcinoma (Table 3).

Photosensitive period of the patient was significantly shortened in the combined PDT as compared to the HPD-PDT

In the combined PDT, the fluorescence spectrum in the tumor lesion showed only a one-peak excitation spectrum before the photosensitizer administration (Fig. 5a). After the topical ALA was applied on the lesion for 4 h on second day following the intravenous injection of the photosensitizer HPD, an emission peak at 630 nm was observed next to the excitation peak in the lesion of the skin carcinoma (Fig. 5b). The emission peak, however, disappeared 4 days after the combined PDT (Fig. 5c). The figures d, e, and f showed photosensitizer levels in the normal skin 2, 4, and 9 days after photosensitizer application, respectively. There was a tiny emission peak at 630 nm in the normal skin 2 days post-administration, but the peak was almost gone 4 and 9 days after the photosensitizer was given (Fig. 5d–f).

The photosensitive period determined by the level of the photosensitizer accumulation in the skin was 31.2 ± 2.5 days for the routine HPD-PDT application, whereas the period in the combined PDT was significantly shortened to 12.3 ± 1.5 days ($P < 0.01$, Fig. 6).

Discussion

In this study, we show that the PDT combined with systemic HPD and topical ALA is used to treat the skin tumors in the head and face. The effectiveness of this new treatment strategy is also compared with those of the traditional HPD-PDT and the ALA-PDT following CO_2 laser vaporization. We find that the combined PDT not only shows high cure rate for skin cancers, which is similar to the ones of the HPD-PDT and the ALA-PDT following CO_2 laser vaporization, but also significantly shortens the photosensitive period to 10–14 days as compared to a 4–5 weeks duration for the traditional HPD-PDT. Therefore, our findings indicate that the combined PDT is an efficient therapy for skin cancers.

The PDD, which is based on the fluorescence ability of the photosensitizer, is a rapid, sensitive, and non-traumatic diagnostic method to detect malignant tumors. It contributes not only to the early diagnosis of the skin cancers but also to fluorescence-guided tumor resection, the evaluation for the efficacy of the PDT, and the judgment of the photosensitive period (Kennedy et al. 1996; Dougherty et al. 1998; Zhu et al. 1999; Kostron 2010; Agostinis et al. 2011). In our present study, the PDD showed the fluorescence intensity of the photosensitizer in the tumor lesion, representing the photosensitizer accumulation in the body, was increased after the administration of the light-sensitive drug.

The HPD is the first photosensitizer used in clinic for the PDD and the PDT (Agostinis et al. 2011). While the HPD is a mixed form of the porphyrin, the pure compound is named as Photofrin. In 1995, the Food and Drug Administration (FDA) of the USA approved the use of the Photofrin in the PDT (Lightdale et al. 1995). The Photofrin has been documented to be used for various types of cancers, such

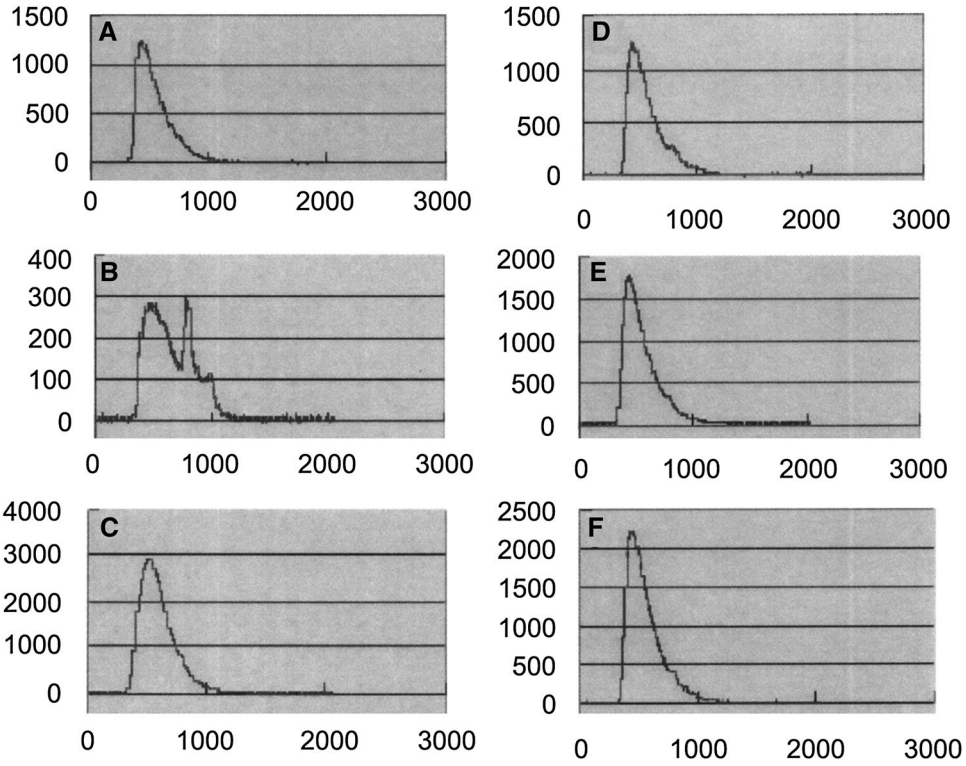

Fig. 5 Fluorescence spectra of the photosensitizer intensity alterations in a cancer lesion after the combined PDT. Before photosensitizer application, the fluorescence spectrum in the tumor lesion only shows an excitation peak (**a**). In contrast, a sharp emission peak at 630 nm, representing the photosensitizer accumulation, is noted in the spectrum after the ALA is applied on the lesion 2 days following the HPD injection (**b**), but the emission peak disappears 4 days after the combined PDT (**c**). In the normal skin, the photosensitizer level is indicated by a tiny emission peak at 630 nm 2 days post-administration (**d**), yet the peak is almost gone 4 and 9 days after the photosensitizer application (**e** and **f**, respectively). **a**–**c** in skin cancer lesions; **d**–**f** in normal skin

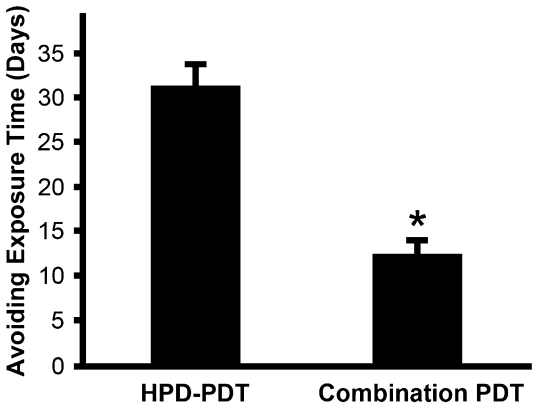

Fig. 6 Reduction of the photosensitive period. The photosensitive period to bright light is significantly decreased in the combined PDT as compared to the one in the traditional HPD-PDT ($p < 0.01$); $n = 28$ subjects for the HPD-PDT and 26 patients for the combined PDT

as cervical, endobronchial, esophageal, bladder, and gastric cancers, as well as brain tumors (Dolmans et al. 2003; Agostinis et al. 2011) and skin cancers (Zeitouni et al. 2001). The remarkable outcomes of the Photofrin-PDT application are achieved (Dugan et al. 1991; Edell and Cortese 1992; Kato et al. 1996). For instance, Dugan et al. (1991) have indicated that the Photofrin-PDT can reduce the cancer recurrence rate from 81 to 31 % over a one-year period after the resection of the papillary bladder cancer. Meanwhile, a study shows that a CR rate of 83.2 % is observed in the PDT with the Photofrin for 95 bronchogenic carcinomas with early stage (Kato et al. 1996).

A compared study of the PDT has been also shown in skin cancer lesions. Zeitouni et al. (2001) have reported that while there is 92 % complete response rate in 6 months after the Photofrin-PDT application is systemically used for 1440 superficial and nodular basal cell carcinomas, the same rate of complete response is also noted in 330 superficial basal cell carcinomas with the topical ALA-PDT. However, the main side effect of the treatment with HPD or Photofrin is phototoxic due to the photosensitizer accumulation in the body after its intravenous administration. Therefore, the patients treated with the classical HPD-PDT have to be kept away from light for 4–5 weeks, which limits the clinical application of the HPD-PDT.

Topical photosensitizer 5-ALA is discovered in the 1990s (Kennedy et al. 1990, 1996; Peng et al. 1997) and is a precursor of protoporphyrin IX (PpIX)—an active photosensitizer. By using heme biosynthetic pathway, the ALA generates endogenous porphyrins, including PpIX (Kennedy and Pottier 1992; Dougherty et al. 1998). Therefore, the administration of the ALA can lead to an accumulation of the photosensitizer PpIX, which can be used for the PDD and the PDT. It has been documented that 87, 5, and 8 % of a total of 826 superficial skin basal cell carcinomas show complete, partial, and no responses to a topical ALA-PDT, respectively, yet the rates of complete, partial, and no responses are 53, 35, and 12 %, respectively, in a total of 208 nodular basal cell carcinomas (Peng et al. 1997).

To get a better treatment for tumors, a repeated ALA-PDT is sometimes needed. For instance, a report shows that the second PDT application can increase the complete response rate of actinic keratosis lesions in two clinical trials (Dougherty et al. 1998). As compared to the complete response rate of 81–86 % after a single treatment, the one after the second treatment is increased to 91–94 %. In our study, we also found an elevation in the complete response rate of the cancer lesions after the second ALA-PDT. In a total of 41 different histological types of skin tumors, while the CR rate of 92.7 % was noted in a single treatment, the second treatment showed 100 % CR rate.

Because being locally applied to the skin lesion, the ALA concentration can be used up to 20 % but has no obvious side effects during and after the ALA-PDT (Dougherty et al. 1998). However, compared with that the HPD-PDT approach can treat a tumor of as deep as 8–12 mm (Agostinis et al. 2011; Zhu et al. 2004), the topical ALA-PDT is generally suitable for the superficial tumors within a depth of 2 mm from the surface, although a deeper penetration of as much as 5 mm has been reported in a mouse model (Casas et al. 2000), which may account for the better outcomes for superficial skin basal cell carcinomas but the poor treatment of the nodular basal cell carcinomas in the ALA-PDT application (Ahmadi et al. 2004) as mentioned above.

As our data showed, the topical ALA dramatically increased the level of the photosensitizer in the cancer lesion, which can contribute to the efficacy of the PDT for the cancers. Meanwhile, the more the photosensitizer is located in the blood vessel, the more the damage to the blood vessels in the tumor seems produced after the PDT (Agostinis et al. 2011). Therefore, when the ALA-PDT is combined with the HPD-PDT, such treatment is able to treat the tumors more effectively. In fact, in our present study, we found the combined PDT showed a higher CR rate for the skin cancers in 2 months as compared to the HPD-PDT did (100 % vs. 92.9 %), although the CR rate in the combined PDT was dropped to 97.6 % during the follow-up period of 6 months to 6 years.Therefore, our findings indicate that the combined PDT is more efficient than the HPD-PDT does in the treatment of different histological types of cancers.

Moreover, in our study, the HPD dose in the combined PDT was reduced to 1.5 from 5 mg/kg in the routine HPD-PDT. Since the photosensitivity is related to the HPD level in the body, the photosensitive period should be shortened in the combined PDT. We actually found that the period is significantly reduced to 10–14 days in the combined PDT compared with a period of 4–5 weeks in the routine HPD-PDT. Meanwhile, as compared to the ALA-PDT, which can treat only superficial tumor with a 2 mm depth, the combined PDT can apply on deeper skin tumors.

The complete or incomplete response of a skin carcinoma to the PDT is related to its characteristics such as the type and the size. For example, in our study, two pigmented basal cell carcinomas showed poor response to the HPD-PDT, which might be because the pigments hinder the light absorption of the tumor cells (Kaviani et al. 2005). However, our finding that these two tumors were cured after the second HPD-PDT indicates the repeated treatments can improve the response rate of the tumor to the PDT, which was also noted in the topical ALA-PDT in our present study.

Conclusions

In this study, our aim was to find a more convenient and efficient PDT strategy for skin cancers. We treated 41 skin cancer lesions with different types in head and face of 26 patients by using the PDT with the application of a topical ALA and a systemic HPD, the efficacy of which was then compared with the one of the topical ALA-PDTs or the systemical HPD-PDT in 41 or 28 skin carcinomas, respectively. We found that the combined PDT not only shows a high cure rate for skin cancers, but also decreases the dose of the pro-toxic HPD and shortens the photosensitive period of the patients, from which patients are able to benefit.

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Compliance with ethical standards

Conflict of interest No competing financial interests exist.

Research involving human participants This clinical study was approved by the board of the Shanghai Jiao Tong University School of Medicine.

Informed consent Informed consent was obtained from all individual participants included in the study. Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

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