Original Research

Investigation of photodynamic therapy optimization for port wine stain using modulation of photosensitizer administration methods

Ying Wang[1], Zhaohui Zuo[2], Xiaohua Liao[3,4], Ying Gu[1], Haixia Qiu[1] and Jing Zeng[1]

[1]Department of Laser Medicine, Chinese People's Liberation Army General Hospital, Beijing 100853, China; [2]Beijing Shuguang Aerospace Electrical Co. Ltd, No. J5 Jingshun Road, Chaoyang District, Beijing 100028, China; [3]Fujian Metrology Institute, Fuzhou 350003, China; [4]Institute of Laser, Fujian Normal University, Key Laboratory of Photonic Technology of Fujian Province, No. 8 Shangshan Road, Cangshan District, Fujzhou 350007, China

Corresponding author: Ying Gu. Email: guyinglaser@sina.com; 25wy@sohu.com

Abstract

To raise photosensitizer concentration level during the photodynamic therapy process, two new methods of photosensitizer administration were investigated. The first method involves the slow intravenous injection of photosensitizer throughout the first 15 min of irradiation, and the second method involves 30 min fomentation before photosensitizer injection and irradiation. The fluorescence spectra of port wine stain skin were monitored and the therapeutic effect correlated index was calculated with a previously published spectral algorithm. Thirty cases were divided into group A (slow injection of photosensitizer during the first 15 min), group B (fomentation), and group C (control group, traditional injection method), with 10 cases in each group. To analyze the effect of these two new methods, the change of therapeutic effect correlated index values of two photodynamic therapy sessions for each patient were calculated, and the photodynamic therapy outcome was compared. The results showed that the change of therapeutic effect correlated index in group A was slightly more remarkable than that in the control group. The change of therapeutic effect correlated index in group B was similar to that in the control group. Slow injection of photosensitizer during photodynamic therapy has a potential to increase photosensitizer concentration level during photodynamic therapy. However, fomentation before photodynamic therapy has no such potential. There is a need for new methods to be attempted.

Keywords: Photodynamic therapy, port wine stains, photosensitizer concentration, fluorescence

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Introduction

Port wine stain (PWS) is a congenital birthmark that is histologically characterized by ectatic vessels within the papillary and reticular dermis. Vascular-targeted photodynamic therapy (V-PDT) with the advantage of dual selectivity effect (selectivity penetration of light in superficial layer of dermis and selectivity photosensitizer [PS] distribution in ectatic vessels) has been applied in the treatment of PWS for 22 years.[1,2] Clinical experiences showed that it is effective for all types of PWS, even for PWS that is resistant to pulse dye laser.[3]

Despite the successful use of V-PDT for PWS, there are still problems that are puzzling doctors. Under the same light dosage and PS dosage, the results of photodynamic therapy (PDT) vary between patients.[4,5] Individuation of V-PDT dosimetry based on real-time monitoring and feedback system is a developing trend in clinical application.[6–8] In clinical application, there is still no available noninvasive light fluence rate monitoring method. Fluorescence monitoring of PS *in vivo* during V-PDT is the most feasible method to monitoring V-PDT procedure.

Inspired by the idea of pharmacokinetic/pharmacodynamic (PK/PD) in pharmacology,[9,10] we have proposed a fluorescence spectral algorithm by which a therapeutic effect-correlated index (TECI) can be deduced. Previous results showed that TECI has a positive correlation with PDT outcome. Patients with TECI values between 11 and 20 usually get blanching grade between II (25–50% clearance of red color) and III (50–75% clearance of red color). It suggested that TECI value is usually required to be above 20 to get a better blanching grade (such as grade II).

This study is an extension of our previously published work.[11] Two PS administration methods were tried to raise the TECI value (i.e. the PS concentration level during PDT). The first method is slow intravenous injection of PS during the first 15 min of irradiation. The second one is 30 min fomentation before PS injection and irradiation. The change of TECI value between two PDT sessions for one patient was calculated to analyze whether the new PS

administration methods have potential to enhance PS concentration during PDT and optimize the PDT effectiveness for PWS.

Materials and methods

Patients

Thirty cases with congenital PWS were recruited in this study. Informed consent was obtained from each patient or his/her guardian. The skin fluorescence spectra were monitored in two consecutive PDT sessions for each patient.

Fluorescence spectrometer

This instrument (Figure 1) is composed of a 440-nm laser diode (B&W TEK Inc., Newark, DE, USA) with a stable output, an optical fiber, and a USB2000 miniature fiber optic spectrometer (Ocean Optics Inc., Dunedin, FL, USA). Fluorescence emission from the tissue was collected by a Y-type fiber, and after suitable filtering (437 + 10 nm), the spectra from 400 to 800 nm were displayed (integration time 4 ms). Acquisition of the spectra was carried out with the software OOIBase32. During measurements, the tip of the fiber was held 45° to the surface of the tissue to avoid the influence of mirror reflection. The acquisition of complete fluorescence emission spectra took less than 1 s, and any PDT effect generated by the excitation light was presumed to be minimal.

Infrared thermometer

Non-contact infrared thermometer was offered by EDRI (The IT Electronics 11th Design Research Institute and Technological Engineering Corporation Limited). Wavelength ranges from 8 to 12 μm. Temperature resolution is 0.05°C, and temperature range is 20–60°C.

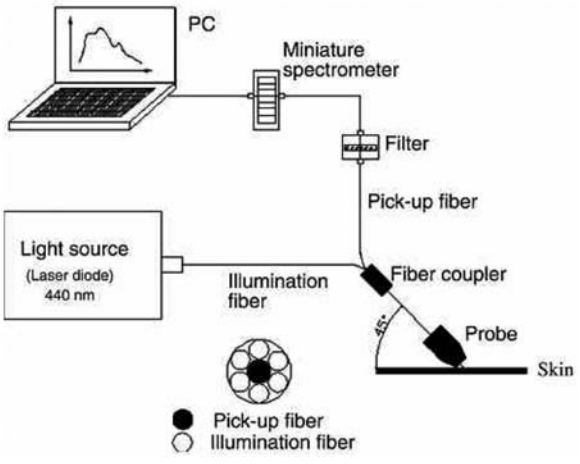

Figure 1 Fluorescence measurement system: 440 nm diode laser, Y-type fiber, filter and computer-controlled spectrometer form the fluorescence measurement system. The measurement arm consists of one pick-up fiber (in the center) and six illumination fibers (at the periphery)

PDT procedure

Domestic PS PSD-007 (Photocarcinorin, provided by the Secondary Military Medical University) and 532 nm LDA laser (Beijing Newraysing Laser Technology Co., Ltd) were applied in PDT treatment. PSD-007 was administered by intravenous injection at a dose of 4–5 mg/kg body weight. Laser power density was 100 mW/cm^2. The laser dosage and PS dosage for each patient was the same in the two PDT sessions.

In the first PDT session, PS was injected intravenously within 2–4 min for all the patients. In the second PDT session, the 30 PWS cases were randomly divided into three groups according to the way of PS administration. In group A, one-third of the PS was injected intravenously in the first 5 min and two-thirds of the PS was slowly injected in the following 10 min of irradiation. In group B, fomentation was performed on the treated area for 30 min, then PS was injected quickly as in the first treatment session. In group C (i.e. the control group), PS was injected intravenously quickly before irradiation as in the first treatment session.

Fluorescence spectra analysis and the calculation of TECI

An algorithm was developed to extract the fluorescence spectra of PS by correcting the distortions imposed by the absorption of melanin and hemoglobin and the autofluorescence spectra of skin from the measured fluorescence spectra. A semi-quantitative concentration of PS was presented with the ratio of PS fluorescence percentage to autofluorescence percentage. To describe the change of PS concentration during the whole treatment, the ratio (FR) was plotted as a function of time and formed one curve for each case. The area under the curve was determined as PDT efficacy indicator and called TECI. The correlation between therapeutic efficiency and the values of TECI had been validated in our previous study.[11]

Evaluation of PDT efficacy

The blanching of the red color of PWS was used as an indicator for PDT efficacy. In this study we used the criteria suggested by Gilchrest *et al.*[12] for the assessment of clinical outcomes of laser therapy. Patients were recorded two or three months after the PDT. Lesion sites were examined visually and photographs were taken before and after treatment. Therapeutic effectiveness was evaluated according to the criteria listed in Table 1.

Statistical analysis

Statistical analysis was performed using SPSS (Statistical Program for Social Sciences) software. Rank-sum test was used to compare the percentage of ΔTECI among the three groups. A difference was considered to be statistically significant with P values < 0.05.

Results

Calculated TECI and treatment outcome of the two PDT sessions in control group

Eight cases completed the follow-up of the two PDT sessions in this group. For most of the cases, the calculated TECI and treatment outcome between two sessions had no significant difference (Table 2; Cases 30, 11, 26, 2, and 24). Their difference of TECI between two sessions ranged from negative 18.53% to positive 13.96%. Table 2 lists the calculated TECI, with the order of increasing percentage ΔTECI.

Only in one case (Case 19), where the patient showed a degree II treatment outcome (more than 50% clearance of red color) in the first session, the TECI reduced 69.88% in the second session. The TECIs of another two cases (Cases 13 and 20) became little higher (43.21% and 22.25%, respectively) in the second session, and their treatment outcome was slightly better than in the first session.

Calculated TECI and treatment outcome of the two PDT sessions in group A

All cases completed the follow-up of the two PDT sessions in this group. Table 3 shows the calculated TECI and treatment outcome of group A. Three cases, as in control group, had a 1.48–50.20% lower TECI in the second session. However, the decrease was smaller than that in the control group. These three cases also had a higher TECI and better outcome in their first session. There was no decrease of treatment outcome in the second session.

Seven cases had a 4.13–187.07% higher TECI in the second session, and three of them showed an increase that was higher than the maximum increase in the control group. The treatment outcome improved in four cases with an increase in TECI of >13%.

Although the results showed that the increase in TECI in group A was greater than that in the control group and the decrease in TECI was less than that in control group, the percentage ΔTECI in group A and control group showed no significant statistical difference.

Calculated TECI and treatment outcome of the two PDT sessions in group B

Seven cases completed the follow-up of the two PDT sessions in this group. In the two sessions, the calculated TECI were all at a low level, and the treatment outcome were only III–IV (Table 4). In the second session, TECI were slightly elevated only in two of the seven cases and decreased in the other five cases. No improvement was observed in the treatment outcome. There was no significant difference in ΔTECI between group B and the control group.

Figure 2 shows the curve of FR to treatment time of Case 6 in two PDT sessions. An increase of PS concentration was observed at the beginning of PDT, but no increase of PS concentration was observed during the whole PDT process.

The average skin temperature of seven patients before and after fomentation was 35.90°C and 41.56°C, respectively. Figure 3 shows the temperature curves of Case 6 during two PDT sessions. In the first session, the skin temperature before laser irradiation was 36.3°C; during the first 3 min, it increased to 41°C and then attained a balance.

Table 1 Criteria of treatment outcome

Grade	Clinical description
I	Become normal skin (75–100% clearance of red color)
II	Slight residual color (50–75% clearance of red color)
III	Obvious lightening (25–50% clearance of red color)
IV	Minimal lightening (~25% clearance of red color)

Table 2 TECI (and treatment outcome) of two PDT sessions in control group

Case No.	First session	Second session	Percentage ΔTECI
19	14.84 (II)	4.47 (III)	−69.88%
30	12.95 (III)	10.55 (III)	−18.53%
11	8.11 (IV)	7.01 (IV)	−13.56%
26	15.95 (III)	15.94 (III)	−0.06%
2	13.10 (III)	14.05 (III)	7.25%
24	7.45 (IV)	8.49 (IV)	13.96%
20	13.98 (III)	17.09 (II)	22.25%
13	13.47 (II)	19.29 (II–I)	43.21%

PDT: photodynamic therapy; TECI: therapeutic effect correlated index.

Table 3 TECI (and treatment outcome) of two PDT sessions in group A

Case No.	First session	Second session	Percentage ΔTECI
18	19.42 (II)	9.67 (II)	−50.20%
17	38.89 (II)	35.53 (III)	−8.64%
8	23.87 (II)	23.54 (II)	−1.48%
9	13.23 (III)	37.98 (II)	187.07%
21	5.65 (IV)	11.79 (III)	108.67%
4	4.48 (III)	7.45 (III–II)	66.29%
22	7.72 (III)	8.79 (II)	13.86%
25	7.25 (III)	7.73 (III)	6.62%
14	16.51 (III)	17.35 (III)	5.09%
1	6.29 (IV)	6.55 (IV)	4.13%

PDT: photodynamic therapy; TECI: therapeutic effect correlated index.

Table 4 TECI (and treatment outcome) of two PDT sessions in group B

Case No.	First session	Second session	Percentage ΔTECI
7	6.76 (IV)	3.48 (IV)	−48.52%
6	8.53 (IV)	5.52 (IV)	−35.29%
16	6.96 (IV)	4.76 (III)	−31.61%
15	13.46 (IV)	9.89 (IV)	−26.52%
29	5.41 (IV)	5.09 (III)	−5.91%
5	6.80 (IV–III)	7.51 (III)	10.44%
10	7.19 (III)	9.15 (III)	27.26%

PDT: photodynamic therapy; TECI: therapeutic effect correlated index.

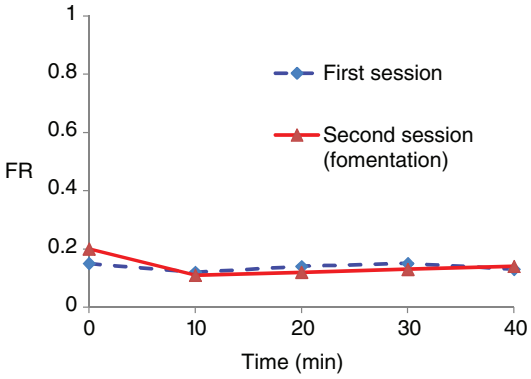

Figure 2 The change of FR during two PDT sessions for Case 6
PDT: photodynamic therapy. (A color version of this figure is available in the online journal)

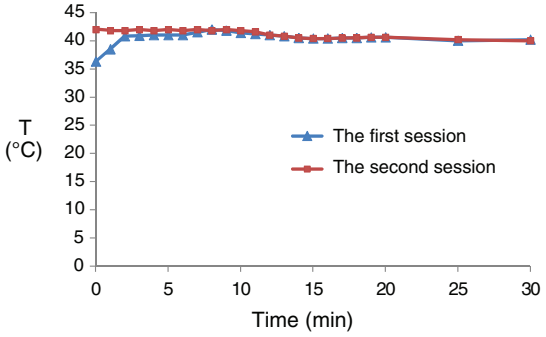

Figure 3 The change of skin temperature during two PDT sessions for Case 6
PDT: photodynamic therapy. (A color version of this figure is available in the online journal)

In the second session, the skin temperature after fomentation was 42.2°C, and there was no evident increase during laser irradiation.

Discussion

V-PDT dosage includes light dosage, PS dosage, and oxygen content. The interaction of light, PS, and oxygen initiates a photochemical reaction that culminates in the generation of highly reactive oxygen species. The latter can rapidly cause damage to vascular endothelial cells and then lead to vascular stasis, thrombus formation, or permanent vessel occlusion. Preliminary experiments showed that the change of oxygen content in skin during PDT did not influence the PDT outcome of PWS.[13] As the ectatic vessels of PWS are superficially located in skin, laser at yellow or green wavelength band can generally penetrate into the target vessels. Therefore, PS concentration in target vessels is one of the most important factors influencing PDT efficiency. Among the available detecting techniques of PS concentration, fluorescence spectra is noninvasive and can be conveniently used in clinics.[14,15] Many researchers had analyzed the correlation between PS fluorescence information in tissue and PDT efficiency, such as PS photobleaching rate as an implicit dosage for PDT treatment outcome of tumor.[16] However, as for PWS, PS photobleaching is not a suitable indicator for PDT efficiency. Because laser irradiation starts immediately after PS injection in V-PDT for PWS, PS concentration in target vessels is markedly influenced by the PKs of PS (such as metabolism and diffusion), besides photobleaching.

As the life time of singlet oxygen (which is the major mediator of photoxic effects of porphyrin-based) is very short (less than 0.5 μs),[17] a stable yield of reactive oxygen species in the target vessels is very essential to obtain a highly efficient treatment. The stable amount of reactive oxygen species can be maintained only when there is enough PS at every time point during V-PDT. However, previous studies suggested that the concentration of PSD-007 decreased notably in the later stage of irradiation.[18] If there was a way to maintain a high level of PS concentration during the whole PDT process, treatment efficiency can be markedly improved, and irradiation time can be shortened. In this study, we attempted new methods of PS administration that may have the potential to increase PS concentration. Slow injection of PS and fomentation before PDT are the most feasible ways in clinics. Here we investigated their effect on PS concentration.

Our results indicated that the higher the TECI value is, the better is the treatment outcome of PDT. This finding is consistent with a previous study.[11] Slow injection of PS during PDT has potential to increase PS concentration level during PDT, whereas fomentation before PDT has no such role.

The ectatic vessels of PWS are mainly located in the papillary dermis. Body conditions (such as temperature, sentiments state, and so forth) have some effects on microcirculation, which includes the diameter and blood flow rate of disease vessels. The change of microcirculation status may lead to the difference of TECI in two PDT sessions. In the control group (group C), there was a fluctuation of TECI and outcome between the two sessions under the same PDT dosage. For patients with a good (degree II or I) treatment outcome after the first session, the amount of ectatic vessels decreased after the first treatment. Their PS content and the calculated TECI reduced in the second session. In this situation, the outcome of the second session was still depended on the TECI value. If TECI was higher, a good outcome could be obtained and vice versa. Take Case 17 as an example; a degree II outcome was obtained after the first session, then a 8.64% decline of TECI was observed in the second session. As the TECI value (35.53) was high, the outcome of the second session was still good (degree II, Figure 4).

In group A, PS was administrated slowly during the first one-third of the time in the second PDT session. The increase of TECI in group A was much more than that in control group (group C), and the decrease of TECI was less than that in the control group. Most cases had a slightly better treatment outcome in the second session, but the improvement was not significant. The possible reason may be that 15 min is too short compared with the whole irradiation time (30–40 min). PS concentration decreased after the first 15 min and maintained a low level. Although the drop in PS concentration was slowed down

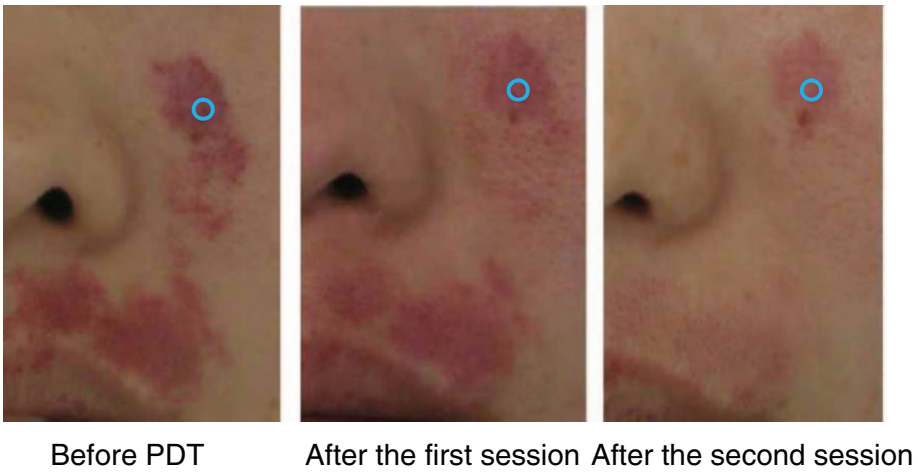

Figure 4 The blanching effect of two PDT sessions in Case17. The blue circles indicate the position where fluorescence spectra were monitored. (A color version of this figure is available in the online journal)

by this method, PS concentration was not increased significantly in the later two-thirds of irradiation time. In view of the small sample size in the present study, a further large-scale study is required to confirm the efficiency of slow injection of PS during the first one half of or the whole PDT irradiation time.

Expansion of capillary vessels can be induced by fomentation.[19,20] An attempt was made to enhance PS concentration in disease vessels using this method, but the results of group B showed that fomentation can only increase the PS concentration at the beginning of irradiation. Laser irradiation itself can induce a temperature increase of 3–9°C in PWS skin during the first 3–4 min of PDT (Figure 4). The effect of fomentation was overwhelmed by laser irradiation when PDT laser irradiation started, and the PS concentration cannot be increased during the whole PDT process. The enhancement effect of fomentation on PS concentration was poorer than that of slow PS injection method.

The present result suggested that fluorescence monitoring method of PS established can provide semi-quantitative information of PS concentration, but the two PS administration techniques tried in this study had no remarkable effect on the increase of PS concentration during the whole PDT process.

This may be attributed to the fast PK rate of PSD-007 in blood. As a second generation PS, PSD-007 has a faster clearance from blood and normal tissues than the first generation PS (hematoporphyrin derivative, HpD). These two techniques used in this study could not contend against the decrease of concentration caused by metabolism. New methods, such as slow-release or controlled-release technology of PS, can be attempted in our future studies.

Conclusions

Slow injection of PS during PDT has potential to increase PS concentration level during PDT. However, fomentation before PDT has no such potential. It is necessary to attempt new methods in future studies.

Author contributions: YG, YW, RC and XHL participated in the study design; YW, XHL and ZHZ conducted the experiments, data collection and analysis of the data; HXQ and JZ assisted with the fluorescence spectra measurement in clinic, RC supplied critical equipment (fluorescence spectrometer); YW wrote the manuscript; YG and HD contributed to manuscript preparation. All authors participated in interpretation of the studies and review of the manuscript.

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