Dose-Related Structural Effects of Photodynamic Therapy on Rabbit Choroidal Structure

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Keywords

Photodynamic therapy · Verteporfin · Dose-dependent effects · Choroidal structure · Central serous chorioretinopathy

Abstract

Introduction: Photodynamic therapy with verteporfin (vPDT) has been shown to be effective against central serous chorioretinopathy (CSC) and was the preferred therapeutic for CSC treatment. However, alterations in choroidal structure after PDT were reported, and these effects were dose-dependent. This study aimed to compare the changes in choroidal structure after PDT with different doses of verteporfin in rabbits and may provide individualized therapeutic guidance for patients who failed to respond to initial half-dose vPDT. **Methods:** The full dose of verteporfin used in CSC was 6 mg/m², which was used in patients with neovascular age-related macular degeneration. Laser fluence was 50 J/cm² (irradiance, 600 mW/cm², 83 s). There were 4 different dose groups in this study (100%, 70%, 50%, and 30%). The alterations were examined at 1 day, 1 week, and 1 month after vPDT using color fundus imaging, indocyanine green angiography, and histopathology analysis. **Results:** Various de-

grees of choroidal alterations were demonstrated at different dose groups. Examinations on day 1 showed that gradually reduced verteporfin dose tended to decrease photochemical reactions to the choroid in terms of the number of occlusion vessels and area of the lesion. After 1 month, choroid vessel alteration persisted in high-dose groups (100% and 70%); nevertheless, alterations of low-dose groups (50% and 30%) returned to normal. **Conclusions:** vPDT can induce photochemical reactions of the choroid, high dose causes permanent change, and low dose causes recoverable change. The dose-dependent alterations need to be considered for the individual therapeutic plan according to the situation of a patient with CSC.

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Introduction

Central serous chorioretinopathy (CSC) is an idiopathic chorioretinopathy that is characterized by serous retinal detachment involving mainly the macular area and accompanied by leakage of altered retinal pigment epithelium (RPE) [1, 2]. Most acute CSC often resolves spontaneously within a few months; however, in some patients,

it may progress to chronic CSC, which can cause photoreceptor degeneration and RPE atrophy, resulting in irreversible anatomical and functional damage [3]. Recently, the understanding of the pathology causing CSC changed from the RPE level to the choroidal disturbance level [4, 5]. It was thought that photodynamic therapy (PDT) with verteporfin could alter choroidal vasculature structure and perfusion, reduce subretinal fluid (SRF), and improve visual acuity for both acute and chronic CSC [6–8].

The dose of verteporfin infusion and laser energy parameters used in CSC were identical to those used in neovascular age-related macular degeneration (nAMD) (6 mg/m², irradiance, 600 mW/cm², 83 s) [9, 10]. It is generally accepted standard therapy regimen in CSC treatment. Although PDT with conventional verteporfin dose yielded favorable effects, post-PDT complications have been reported in patients, such as RPE atrophy, RPE tear, secondary choroidal neovascularization, and choroidal ischemia [11–13]. As a result, many studies on modified PDT protocols have been evaluated, including lowering verteporfin doses, decreasing laser power, decreasing laser treatment times, or shortening the treatment interval [14–17]. Some studies showed that 50%-dose PDT has favorable results compared with full-dose PDT [14, 18, 19], but there remain failed cases after the initial half-dose PDT. Data on the treatment regimen for patients without response to initial half-dose PDT are insufficient due to the paucity of studies. It has been verified that PDT retreatment for patients with recurrence after failure of initial PDT is effective [20, 21]. The optimum dose for retreatment should be established; therefore, experimental evidence is needed to clarify the impact of multiple drug applications on tissue construct.

Nevertheless, few animal or clinical studies have compared the photochemical reactions of choroid vessels caused by PDT using different doses of verteporfin. This study aims to evaluate choroid vessel structure changes caused by PDT using different doses of verteporfin contributing to treatment response on choroidal vessels in rabbits. The results of this animal study are crucial for not only determining therapeutic effects but also preventing future complications in individualized CSC treatment.

Fig. 1. Color fundus image. **a1** Control group showed normal fundus. **a2** Verteporfin-only group. **a3** Laser-only group showed normal fundus 1 day after treatment. **b1–b3** Fundus photographs of the 100% dose group. **c1–c3** Fundus photographs of the 70% dose group. **d1–d3** Fundus photographs of the 50% dose group. **e1–e3** Fundus photographs of the 30% dose group. Retinal detachment and transient SRF were noted 1 day after treatment in all treatment

Materials and Methods

Animals

Adult Chinchilla bastard rabbits (*Oryctolagus cuniculus*, standard Chinchilla) of either sex weighing 2.5–3.0 kg were used for the experiments (obtained from Beijing Tiantan Biological Products Co., Ltd., Beijing, China). Rabbits were maintained at the Animal Laboratories of the Peking University People's Hospital. Animals were used in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Peking University People's Hospital (permit No. 2014-17).

A total of 33 rabbits were included in this study. They were randomly divided into 7 groups: normal control (3 rabbits), verteporfin only (3 rabbits), laser only (3 rabbits), 100% dose (6 rabbits), 70% dose (6 rabbits), 50% dose (6 rabbits), and 30% dose (6 rabbits). Rabbits were anesthetized using 3% isoflurane inhalation via a facemask. Pupils were dilated with 1% atropine sulfate ophthalmic gel (Xingqi Pharmaceutical Co., Ltd., Shenyang, China), 0.5% tropicamide, and 0.5% phenylephrine hydrochloride eye drops (Santen Pharmaceutical Co., Ltd., Osaka, Japan).

After PDT, rabbits were kept under dark conditions for 3 days to prevent light damage. Then, the animals were euthanized with sodium pentobarbital overdose, and the eyes were enucleated for analysis.

PDT Procedures

The currently accepted standard dose of verteporfin used in CSC was identical to the dose used in neovascular age-related macular degeneration patients (6 mg/m²) [9, 10]. Verteporfin (Visudyne, Novartis, AG, Bulach, Switzerland) was used according to the manufacturer's instructions at a concentration of 2 mg/mL and dissolved in 7 mL of sterile water; the bottle was protected from light and used within 2 h. The 100% dose of verteporfin used in rabbits was 0.43 mg/kg [22], which was extrapolated from the recommended dose in humans of 6 mg/m². Moreover, 70% dose was 0.301 mg/kg, 50% dose was 0.215 mg/kg, and 30% dose was 0.129 mg/kg.

For PDT lesions, a diode laser at 689 nm with a slit-lamp delivery system (Opal Photoactivator, Lumenis Inc, Santa Clara, CA, USA) was used. Marginal ear vein infusion of verteporfin was performed for 10 min, followed by laser delivery at 15 min from the start of infusion. The effect of laser illumination was evaluated at a fluence of 50 J/cm² (irradiance, 600 mW/cm², 83 s). A factor of 0.66 for the rabbit eye was used [23], and the diameter of the laser spot was 2,500 µm. Two laser spots were placed at a distance of 1/2 papillary diameter from the inferior optic disc margin. All rabbits received PDT in 1 eye, and the other eye did not receive any treatment. To ensure consistency, PDT was performed by an experienced ophthalmologist.

groups (**b1–e1**); 1 week after treatment, the 100% dose and 70% dose groups still had retinal detachment and SRF (**b2, c2**); retinal detachment and SRF disappeared in the 50% dose and 30% dose groups (**d2, e2**); 1 month after treatment, RPE mottling was observed in the 100% dose and 70% dose groups (**b3, c3**); retinas of the 50% dose and 30% dose groups returned to normal (**d3, e3**). SRF, subretinal fluid. (For figure see next page.)

Color Fundus Photography and Indocyanine Green Angiography

Color fundus photography (Optomap, Optos plc, Scotland, UK) and indocyanine green (ICG) angiography (Heidelberg Tomograph-HRT II, Heidelberg Engineering, Heidelberg, Germany) were performed on both eyes of all rabbits at 1 day (1 D), 1 week (1 W), and 1 month (1 M) after PDT. The anesthetized animals were injected with 0.5 mL ICG (5 mg/mL, Dandong Yichuang Pharmaceutical Co., Ltd., Liaoning, China) via the ear vein. The hyperfluorescent area indicated damage to the choriocapillaris, and hypofluorescent area inside the laser lesion indicated vessel occlusion.

Histopathology

Eyes were enucleated fixed in 4% paraformaldehyde overnight at 4°C at 1 D, 1 W, and 1 M after PDT. The anterior segments were removed, and the posterior eyecups were embedded in paraffin, sectioned at 5 µm, deparaffinized, rehydrated, and stained with hematoxylin and eosin (H-E). Section image analyses were performed using an Olympus microscope (Olympus, Tokyo, Japan).

Electron Microscopy

The rabbits were sacrificed, and the eyes were collected and fixed in 3% glutaraldehyde (Electron Microscopy Sciences, Hatfield, PA, USA) at an appropriate time point. Then, the specimens were postfixed with 1% osmium tetroxide (Electron Microscopy Sciences, PA, USA), dehydrated, and embedded in Epon (Eponate 12, Ted Pella, Redding, CA, USA). The retinal sections were obtained, contrasted with uranyl acetate and lead citrate, and analyzed using a Hitachi transmission electron microscope (Hitachi, Tokyo, Japan).

Results

Color Fundus Imaging Changes after PDT

In the verteporfin-only and laser-only groups, the fundus appearance was unchanged 1 D after treatment (Fig. 1a2, a3); however, various degrees of chorioretinal responses were demonstrated in different dose groups. Retinal detachment and transient SRF were gradually decreased from 100% dose group to 50% dose group; in 30% dose group, only slight retinal whitening corresponding to the laser irradiation spot appeared (Fig. 1b1–e1). After 1 W of treatment, 100% dose and 70% dose groups still

had retinal detachment and SRF, but the detachment area was shrunk, and the SRF was mostly or completely absorbed (Fig. 1b2, c2). Retinal detachment was not observed in 50% dose group, and pigmentation appeared (Fig. 1d2). In 30% dose group, whitening of the lesions was barely observed in the treated sites (Fig. 1e2). Retinas of 100% dose and 70% dose groups diffused with hyper- or hypopigmented patch and RPE mottling were intermingled at 1 M after PDT (Fig. 1b3, c3). Retinas of 50% dose and 30% dose groups returned to normal (Fig. 1d3, e3).

ICG Angiography Changes

One feature common to vPDT response after 24 h was the presence of a circle of choroidal hyperfluorescence corresponding to the laser spot size and a hypofluorescent patch area inside the laser lesion, suggesting more severe chorioretinal damage and representing vessel occlusion. The higher the verteporfin dose, the larger the hypofluorescent area inside the laser lesion (Fig. 2b1–e1).

Seven days following treatment, gradual reappearance of patent choroidal vessels was observed; however, hypofluorescence was still observed in the center of the laser lesions, with a hyperfluorescent ring in the periphery in the 100% dose and 70% dose groups (Fig. 2b2, c2). This pattern remained constant during all phases of the angiography. In the 50% dose group, persistence of hypofluorescence of the lesion center was observed, while the hyperfluorescence intensity of the surrounding ring markedly decreased (Fig. 2d2). The 30% dose group did not show any alterations in choroid vessels (Fig. 2e2).

At 30 days after treatment, reperfusion of the occluded choroid vessels occurred in 100% dose and 70% dose groups. There were still small areas of hypofluorescence, which demonstrated persistence of perfusion defects in choroid vessels (Fig. 2b3, c3). No angiographic aspect of the choroid vessel was observed in the 50% dose and 30% dose groups (Fig. 2d3, e3).

Fig. 2. ICG angiography. **a1** Control group showed normal ICG angiography image. **a2** Verteporfin-only group. **a3** Laser-only group exhibited normal choroidal vessel appearance without leakage or occlusion. **b1–3** ICG angiography of the 100% dose group. At 1 day after PDT, hyperfluorescence corresponding to the laser spot size and hypofluorescent patch inside the laser lesion were noted; at 1 week after PDT, hypofluorescence still in the center of the laser spots, with a hyperfluorescent ring in the periphery. At 1 month after PDT, choroid vessels showed reperfusion, but existed in small areas of hypofluorescence; ICG angiography of 70% dose group (**c1–**

3). Angiography findings were consistent with 100% dose group. **d1–3** ICG angiography of 50% dose group. At 1 day after PDT, the choroid presented hyperfluorescence ring, and hypofluorescent patch area inside the laser lesion was noted. At 1 week after PDT, hypofluorescence was observed, while the hyperfluorescence ring decreased. At 1 month after PDT, choroid vessels were normal; ICG angiography of the 30% dose group (**e1–3**). At 1 day after PDT, angiography findings were consistent with those of the 50% dose group. At 1 week after PDT, no alteration of choroid vessels was observed. ICG, indocyanine green; PDT, photodynamic therapy.

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Histopathologic Findings

Histologic examination of laser-only group and verteporfin-only group showed a normal pattern without any observable pathologic change in the retina and choroid tissue at 1 D after treatment (Fig. 3a–c, Fig. 4a–c). Light microscopy of H-E staining and electron microscopy showed a pattern of milder photochemical reaction to the choroid and retina structures with decreasing verteporfin dose levels. The histological changes were mainly the same between the first and second irradiation spots in the same eye. H-E staining sections of 100% dose and 70% dose groups revealed not only thrombosis of medium and large size choroidal vessels but also destruction of the RPE and OS (outer segment) and large amount of subretinal exudation 1 D after irradiation. On day 7 after treatment, choroidal vessel thromboses nearly resolved, a small amount of exudation remained, and RPE cell layer discontinuity did not recover. At 1 M, the blockage of choroidal vessels almost disappeared, choroid fibrous proliferation and exudation resolved, and there was thinning of the outer nuclear layer (Fig. 3d–e).

In the 50% dose group, 1 D after PDT, distended choroidal vessels and very few obstructed choriocapillaries can be detected, and modest disruption of RPE, photoreceptor cell layer, and intermediate subretinal exudation were observed. At 1 W after PDT, dilatation of the choroid vessels was observed, and RPE cell discontinuity did not recover. After 1 M, choroid and retina structures returned to normal (Fig. 3f).

Histological damage in the 30% dose group was milder than that in the 50% dose group. Small but visible exudation, distended choroidal vessels, and fewer obstructed choriocapillaries were detected in H-E staining section on day 1 after PDT. After 1 W, dilatation of the choroid vessels was observed, and other tissues recovered to normal. At 1 M, both choroid and retina structure returned to normal (Fig. 3g).

On electron microscopy, intact choriocapillary microstructure was detected in the verteporfin-only and laser-only groups compared with the control group (Fig. 4a–c). All PDT with verteporfin groups showed damage to choriocapillary microstructure 1 D after PDT. Bruch's membrane showed discontinuity, and choriocapillary endothelial cell displayed severe swelling. In the vessel lumens, monocytes and hemolyzed red blood cells were observed (Fig. 4d–g). The degree of severity differed among the 4 groups. The 100% dose group (Fig. 4d) and the 70% dose group (Fig. 4e) had more occluded vessels, and the response degree was milder in the 50% dose group (Fig. 4f) and the 30% dose group (Fig. 4g). The 30% dose group

also showed less severe condition than the 50% dose group. At 1 W after PDT, the choriocapillary in 100% dose and 70% dose groups showed rough basal lamina, and the fibrin was visible in the vascular lumina and the surrounding tissue. The choriocapillary microstructure of 30% dose and 50% dose groups almost returned to normal. On 1 M after laser irradiation, abnormalities of the vascular lumen, basal lamina, and endothelial cell were still prominent in 100% dose group and 70% dose groups, and the basal lamina was still lightly rough and had occasional discontinuity. The other 2 groups showed a normal vascular microstructure.

Discussion

PDT was originally used as a clinical therapy for malignant tumors. In 2003, it was proposed for patients with CSC. Previous studies have demonstrated beneficial visual outcomes in most patients [6, 24, 25]. One limitation of the current PDT treatment was the damage to normal structures. Additional adverse effects to the normal retina, RPE, and choroid caused by PDT are dose-dependent [26, 27]. To prevent adverse effects, half-dose or low-fluence PDT has been studied in the treatment of CSC. A retrospective study conducted by Nicolo et al. [28] revealed that, although half-dose PDT and half-fluence PDT had equal visual improvement and safety in long-term follow-up, half-dose PDT had the advantage of a more rapid reabsorption of the fluid and lower recurrence rate. Decreased fluence PDT might cause more significant inflammatory reaction or increase collateral damage to the normal tissue. To improve treatment safety in CSC, half-dose PDT was proposed. The resolution rates of the SRF after half-dose PDT vary from 60% to 95% [29–31]. Our previous study evaluated a range of verteporfin doses from 10% to 70% and found that 30% was the lowest effective dose, while 50% was better than a 30% with SRF completely resolved in 95% of patients [32].

However, recurrence of SRF occurred in some patients (10–20%) after initial 50%-dose PDT [33]. These patients required re-treatment. It is believed that patients with persistent SRF for at least 3 months since the previous PDT should benefit from re-treatment, especially if there has been a positive response [34]. To the best of our knowledge, presently, the re-treatment strategy for eyes with persistent SRF did not have uniform standard. The application of higher dose vPDT in patients requiring re-treatment leads to a better therapeutic efficacy but may

Fig. 3. H-E staining of rabbit choroid vessels. **a** Control group: normal rabbit retina and choroid. **b** Verteporfin-only group. **c** Laser-only group showed a normal pattern without any pathologic change. **d** In the 100% dose group, at 1 day after PDT, vessel closure of choriocapillaries and deeper choroidal vessels were observable (arrows), OS layer condensed, RPE destroyed, and SRF (*) was damaged. At 1 week after PDT, choriocapillaries thromboses nearly vanished, RPE cell layer showed discontinuity, and a small amount of exudation remained. At 1 month after PDT, choriocapillary occlusion disappeared, and choroid fibrous proliferation and exudation resolved. **e** In the 70% dose group, at 1 day after PDT, the same was found. **f** In the 50% dose group, at 1 day after PDT, distended choroidal vessels, few obstructed choriocapillaries (arrows), and mild SRF (*) were observed. At 1 week after PDT, dilatation of the choroid vessels was observed, and RPE cells discontinuity did not recover. After 1 month, choroid and retina structure returned to normal. **g** The same was found in 30% dose group, but the damage was milder than that in the 50% dose group. GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; OS, outer segment; RPE, retinal pigment epithelium; SRF, subretinal fluid; H-E, hematoxylin and eosin; PDT, photodynamic therapy; arrow, occluded choroid vessel; *, SRF; scale bar, 50 μ m.

also result in potential adverse events. It is important to minimize potential retinal toxicity during PDT while maintaining the treatment effects. The complex structural changes of the chorioretinal anatomy that limits in vivo imaging, such as optical coherence tomography and ICG angiography, indicated that much of our understanding of this critical structure has originated from histopathological analysis.

Recently, the new imaging technique of optical coherence tomography angiography (OCTA) has improved the visualization of the chorioretinal vascular structures and microcirculation in vivo [35]. Demircan et al. demonstrated choriocapillaris alterations using OCTA after half-fluence PDT at 3 days and 30 days following therapy. They concluded that there was a markedly decreased choriocapillary flow limited to the site of the PDT spot in the very early period following PDT in eyes with CSC, and the choriocapillary perfusion returned to normal at day 30 [36]. In the study by Alovisi et al. [37] measured by OCTA scan, half-dose PDT seems to produce short-term (1 week after PDT) changes on the luminal component of both the choriocapillary and choroid, which returned to normal status at 1 month from treatment. Although OCTA has proven to be a valuable tool for the depth-resolved evaluation of the retinal and choroidal structures, the structural changes of choriocapillary and choroid tissue after PDT are still unclear.

Normal choriocapillary and choroid structure changes after PDT are unavailable in humans. Our observation represents a novel finding in an animal model. Even 30%-dose vPDT yielded minimal choroid change, as choroidal hyperfluorescence corresponding to the laser spot size and a hypofluorescent patch area inside the laser lesion on fundus photography and ICG angiography were observed. Histopathology showed distended choroidal vessels, obstructed choriocapillaries, and small but visible exudation 1 day after PDT. Choroidal changes aggravated upon increasing verteporfin dose. Until the end of our observational period (1 month after treatment), we continued to observe choroid hypofluorescence and some

pigment mottling in the RPE in high-dose groups (70% dose and 100% dose) on fundus photography and ICG angiography, but low-dose groups (30% dose and 50% dose) recovered to normal. Previous clinical studies showed that PDT works by producing temporary and reversible choriocapillary hypoperfusion or occlusion. Our findings are in agreement with these observations. We also confirmed that photochemical reactions in PDT on the choriocapillary and choroid are also dose-dependent.

It is well known that cytotoxicity in PDT mainly results from the production of high reactive singlet oxygen (1O_2) or oxygen radical ions, which is generated from the reaction of photosensitizer promoted to an excited state under light [38]. Oxygen radicals specifically act on vascular endothelial cell and lead to vascular endothelial cell alteration. Consequently, subretinal exudation was detected after treatment. Under different levels of verteporfin dose, the degrees of choriocapillary alteration are different. Therefore, it might be speculated that with low verteporfin dose, the effect of PDT is just small endothelium lesions (angiographic hyperfluorescence without vessel occlusion) rather than vessel clotting, so the initiation of a 50% dose might be too low to have a significant effect in some patients. With increasing verteporfin dose, the alteration in choroid vessel increased, may further promote vascular occlusion or thrombosis (angiographic hypofluorescence), and plays a positive role in patients requiring re-treatment. However, it may potentially cause side effects when the verteporfin dose was extremely high.

A human clinical trial reported full-dose vPDT, revealed choroidal hypoperfusion with choriocapillary destruction at 2 years of observation [39], and suggested permanent closure of part of choroidal vasculature. Some clinical studies confirmed the 30% dose seemed to be safe and effective in CSC treatment; therefore, successful rate in the 30% dose was < that in the 50% dose, and recurrence rate in the 30% dose group was > the 50% dose group [32, 40, 41]. Thus, in our clinical work, patients with failure to initial half-dose treatment required re-treatment with the same dose or gradually increased

Fig. 4. Electron microscopy appearance of rabbit choroid vessels. **a** Control group. **b** Verteporfin-only group. **c** Laser-only group. **d** 100% dose group. **e** 70% dose group. **f** 50% dose group. **g** 30% dose group. Intact choriocapillary microstructure was detected in verteporfin-only and laser-only groups compared with that in the control group. At 1 day after PDT, all verteporfin PDT groups showed damage to choriocapillary microstructure. Bruch's membrane showed discontinuity (arrow heads), and choriocapillary endothelial cell displayed severe swelling. In the occluded vessel lumens (*), cell fragments were observed. At 1 week after PDT, in the 100%

dose and 70% dose groups choriocapillary showed rough basal lamina, and cell debris was visible in the vessel lumen. Choriocapillary microstructure of the 30% dose and 50% dose groups almost recovers to normal. At 1 month after PDT, abnormalities of vascular lumen and endothelial cell were still prominent in the 100% dose and 70% dose groups. Moreover, the 50% dose and 30% dose groups appeared a normal vascular microstructure. En, endothelial cell; Pe, pericyte; RPE, retinal pigment epithelium; OS, outer segment; PDT, photodynamic therapy; arrowhead, Bruch's membrane; *, vessel lumen; scale bar, 2 μ m.

(For figure see next page.)

verteporfin dose. In the retrospective study of Oh et al. [42], patients with relapse were received additional treatment, all had a good clinical prognosis with a decrease in or a complete absorption of SRF, and none of them had ocular or systemic adverse events. The dose of re-treatment was not reported in their study. However, through histological experiments, we showed that more serious tissue damage occurred in the higher dose group. Observations of the PDT effect on the choroid tissue are valuable for treatment selection in clinical practice. Eyes with persistent SRF or recurrence after initial half-dose PDT, they needed additional re-treatment. It is crucial for patients to select the appropriate re-treatment option. Therefore, we advocate starting with low dose and escalating to full dose based on treatment response in clinical PDT.

Our study has several limitations, including small sample size and short-term follow-up period. To avoid more sacrifice, we used 6 rabbits for each group. Moreover, the adverse effect of PDT on chorioretinal function should be observed after treatment of different doses.

In summary, PDT with verteporfin used clinically in the treatment of CSC induces injury of the physiological choroid, high dose may cause permanent alteration, and low dose causes slight change. The ophthalmologist should make individualized treatment plans according to patients' situation, especially for those patients who failed in the initial PDT and required multiple re-treatments. This will be beneficial for patients to reach optimal therapeutic effect and diminish side effects.

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Statement of Ethics

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All experimental procedures performed in studies involving animals were in accordance with the ethical standards of Institutional Animal Care and Use Committee of Peking University People's Hospital (permit No. 2014 – 17).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Wei Du wrote this manuscript. Mingwei Zhao provided the idea of this study, performed PDT, and revised this manuscript. Wei Du, Yin Chih Lee, Tianfu Wang, Haoran Cui, Hui Xu, Xuan Bao, and Xin Tang performed the experiment process. All the authors have read and approved the final manuscript.

Data Availability Statement

All data generated during this study are included in this article. Further enquiries can be directed to the corresponding author.

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