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Chlorin e6 as a Photosensitizer for Inducing Targeted Effects in Transscleral Photodynamic Therapy

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Abstract

Introduction: This study aimed to evaluate the role of chlorin e6 in mediating targeted vascular injury during transscleral photodynamic therapy (TS-PDT) under controlled thermal conditions.

Methods: Twenty eyes of ten rabbits were randomized into two groups. The TS-PDT group received intravenous chlorin e6 (2.5 mg/kg) followed by transscleral laser irradiation (660 nm, 0.17 W, 10 min.). The control group (TSLT) underwent identical laser exposure without a photosensitizer. Scleral temperature was monitored in real time using a thermal probe. On day 14, histological analysis assessed choroidal vascular thrombosis and stromal fibrosis using a semiquantitative scoring system. Retinal and scleral tissue damage was also evaluated.

Results: The analysis accounting for inter-animal clustering revealed a significant association between chlorin e6 administration and choroidal vascular thrombosis (90% vs. 50%; adjusted OR = 9.8, 95% CI: 1.4–68.9; $P = 0.021$) and fibrosis (80% vs. 40%; adjusted OR = 6.2, 95% CI: 1.1–35.0; $P = 0.039$). Severity scores for thrombosis and fibrosis were also significantly higher in the TS-PDT group ($P < 0.001$ for both). No significant differences in scleral temperature ($P \geq 0.45$) orretinal/scleral damage were observed.

Conclusion: Vascular damage during TS-PDT is primarily photochemical, mediated by chlorin e6. No significant intergroupdifferences were observed in monitored scleral surface temperaturesunder these parameters, arguing against a major contribution from differential surface heating. The method demonstrates short-term structuralpreservation of surrounding ocular tissues, supporting itspotential as an organ-preserving treatment for intraocular tumors.

Keywords: transscleral photodynamic therapy, chlorin e6 photosensitizer, uveal melanoma, ocular oncology, choroidal melanoma

Introduction

Transscleral photodynamic therapy (TS-PDT) is a promising organ-preserving treatment for uveal melanoma (UM), allowing laser irradiation to reach the tumor base through the sclera and impact both vascular and tumor structures.[1-10] Its effectiveness depends on a true photochemical mechanism, which fundamentally differs from nonspecific thermal injury seen with transscleral laser exposure in the absence of a photosensitizer (PS).[11]

To confirm a genuine photodynamic effect, comparative studies with a control group receiving identical laser parameters but without a PS are essential.[12-20] This design isolates nonspecific factors - such as baseline heating or chromophore absorption - and attributes tissue changes specifically to photochemical reactions involving the activated PS. This methodology is standard in PDT research across models from infection control to oncology.[12-20] For instance, Funjan M.M. (2022) found no significant microbial inhibition from laser exposure alone without a PS.[18] Studies on lightly pigmented tissues (e.g., skin, meninges)

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suggest that lasers in the PDT spectrum may cause mild, non-critical heating even at safe exposure levels.[13,19] This is especially relevant in ophthalmology, where highly pigmented tissues like the retinal pigment epithelium or uveal melanoma may influence energy absorption, yet their thermal response remains underexplored.

Second-generation chlorin-based PSs, particularly chlorin e6, show high potential for TS-PDT. Unlike first-generation porphyrins, chlorins have an optimized absorption peak (~660 nm), enabling deeper penetration with lower absorption by melanin and hemoglobin.[5,21,22] However, melanin's high absorption at this wavelength still poses a risk of localized overheating, necessitating strict thermal control even at safe surface settings.

Temperature regulation is critical for maintaining the photochemical nature of PDT.[19,20] Temperatures exceeding 40–42 °C impair PS function, reduce singlet oxygen ($^1O_2$) generation by 50–70%, promote hypoxia, and shift the balance toward coagulative necrosis.[11,20,23,24] Thus, energy parameters must be carefully balanced - sufficient to activate the PS in deeper layers without triggering thermal damage. Subtherapeutic settings result in inefficacy, while excessive parameters risk PS destabilization and collateral injury. Supporting this, Orlova et al. (2022) showed that blue light (405 nm) caused more heating (~5 °C) than red light (660 nm) due to endogenous chromophore absorption.[20] This emphasizes the need for real-time temperature monitoring, especially in deeper tissues and when using shorter wavelengths.

Laser activation of the PS induces selective vascular injury, leading to thrombus formation, organization, and fibrosis. This vascular selectivity - absent in thermal exposure - is a major advantage of PDT. Therefore, evaluating the role of chlorin e6 in inducing vascular damage under non-thermal conditions is scientifically and clinically essential. The present study, with a control group design, was specifically developed to address this objective. The aim of this study was to evaluate the role of chlorin e6 in mediating targeted vascular injury during transscleral photodynamic therapy under controlled thermal conditions.

Materials and Methods

The study was conducted on 10 healthy rabbits (20 eyes), each weighing 3.0–3.5 kg, selected based on the results of a preliminary ophthalmic examination. The animals were housed under standard vivarium conditions with regulated lighting, optimal ambient temperature (22 ± 2 °C), and relative humidity (50 ± 10%). The experiment was performed in accordance with the principles of the Declaration of Helsinki and the European Directive 2010/63/EU. The protocol was approved by the local ethics committee of the Federal State Budgetary Scientific Institution "IEM" (Approval No. 4/24 dated 24 October 2024). All procedures were performed under general anesthesia, and all efforts were made to minimize animal suffering.

Chlorin e6 (commercially available as «Photolon», Belarus) was used as the photosensitizer at a dose of 2.5 mg/kg body weight. It was administered intravenously via the marginal ear vein 30 minutes prior to laser irradiation. This agent was selected due to its high selectivity for accumulation in the choroidal vasculature, its efficient singlet oxygen ($^1O_2$) generation, and its extremely low toxicity - even at doses substantially exceeding therapeutic levels - well-documented in numerous preclinical studies.[2,4,25-27] Considering species-specific differences in metabolism and pharmacokinetics in small laboratory animals, the applied dose (2.5 mg/kg) substantially exceeds the clinically recommended dose for humans (0.8–1.2 mg/kg), which reflects the faster metabolic rate and shorter tissue accumulation window in rabbits. Likewise, the shortened interval between PS injection and laser exposure (30 minutes versus 3 hours typically recommended in humans) was based on previously published pharmacokinetic studies in rabbits, demonstrating rapid distribution and clearance of chlorin e6.[28,29]

TS-PDT was performed under general intramuscular anesthesia using a combination of Zoletil (50 mg/mL, 1 mL/kg) and Rometar (20 mg/mL, 0.3 mL/kg). A 660-nm diode laser ("ALOD-01," Alkom Medika, Russia) served as the irradiation source. The laser operated in a continuous-wave mode. Prior to each experimental session, the laser output power was calibrated at the tip of the transscleral probe using an optical power meter to ensure the accuracy of the delivered power density. Laser delivery was carried out using custom-designed 5-mm diameter transscleral probes equipped with integrated thermal sensors for continuous real-time scleral temperature monitoring and a vacuum fixation system. The probes featured a flat contact element to ensure firm and uniform application to the scleral surface. The embedded temperature sensors (thermoelectric and thermoresistive) were positioned at the tip in direct contact with the ocular surface. The vacuum fixation system ensured stable, consistent, and minimally compressive apposition to the scleral surface during irradiation. This probe design is protected under Russian Federation Patent No. 2025105194. A schematic illustration of the probe is provided in Figure 1.

The animals were evenly distributed between two groups. The first group consisted of 5 rabbits (10 eyes) that underwent TS-PDT following intravenous administration of the photosensitizer chlorin e6. The second group also included 5 rabbits (10 eyes) but received transscleral laser irradiation without photosensitizer injection (transscleral laser therapy, TSLT). Laser parameters were standardized and identical across both groups: probe diameter 5 mm; output power 0.17 W, corresponding to a power density of 0.866 W/cm² and an energy density of 519.5 J/cm² or an exposure duration of 600 seconds (10 minutes) (reported as the incident energy at the scleral surface). Based on prior ex vivo measurements of 660-nm light transmission through rabbit sclera, the estimated irradiance at the choroidal level was approximately 0.43 W/cm², corresponding to an estimated fluence of 260 J/cm². These parameters were selected based on prior in vitro studies demonstrating a twofold attenuation of laser power upon transmission

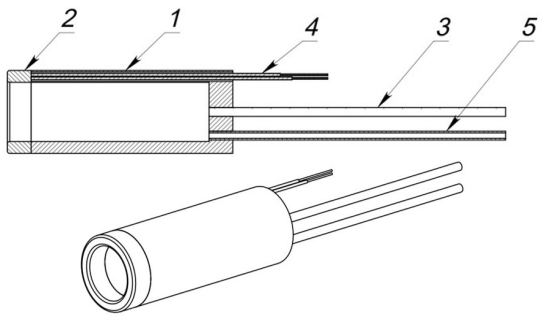

Figure 1. Schematic illustration of the custom transscleral probe with an integrated thermal sensor and vacuum fixation system. One variant of the device implementation is shown in a longitudinal cross-section. Key components include the probe body (1), a ring-shaped thermal sensor (2) for real-time temperature monitoring, an optical waveguide (3) for delivering laser irradiation (660 nm), and a channel (5) for creating a vacuum to ensure stable and uniform apposition of the tip to the scleral surface

through cadaveric sclera.[30] Additionally, the selected values align with recommended ranges for in vivo PDT (power density 0.3–2.0 W/cm², energy density 50–300 J/cm²).[31-33] These parameters are also supported by a recent study determining the therapeutic window for TS-PDT with chlorin e6.[34] The increase in initial irradiation parameters was designed to compensate for inevitable energy losses due to light scattering and absorption in biological tissues, thereby ensuring that a therapeutically effective dose reached the deeper ocular layers.

Temperature monitoring was performed in real time, with measurements recorded at three time points: immediately before irradiation ("baseline temperature"), after 5 minutes ("T at 5 min"), and after 10 minutes of exposure ("T at 10 min"). The temperature change (ΔT, °C) was calculated as the absolute difference between the final and initial temperature values, reflecting tissue heating dynamics during the procedure.

For histological examination, enucleated globes were fixed in 10% neutral buffered formalin, processed using isopropyl alcohol dehydration (Sakura VIP6 tissue processor), embedded in paraffin, and sectioned at 4 μm using a Sakura SRM200 microtome. Hematoxylin and eosin (H&E) staining was performed using an automated system (Sakura Tissue-Tek Prisma Film). For each eye, three full-thickness histological sections through the center of the irradiation zone were evaluated. Morphological changes were evaluated using a custom semi-quantitative scoring system (0-4) applied by two experienced pathologists who were blinded to group allocation. The scoring criteria were as follows: for choroidal vascular thrombosis: 0 = none; 1 = isolated, non-occlusive thrombi in capillaries/small venules; 2 = multiple small thrombi or partial occlusion in medium-sized vessels; 3 = large, occlusive thrombi in several medium to large vessels; 4 = extensive, confluent thrombosis in multiple large vessels; for choroidal stromal fibrosis: 0 = normal stromal architecture; 1 = mild, focal increase in stromal density without architectural disruption; 2 = moderate, multifocal stromal thickening and collagen bundle condensation; 3 = marked, diffuse stromal expansion with hyalinization and clear loss of normal vascular channels; 4 = severe, sclerotic scar tissue replacing the normal choroid. Discrepancies were resolved by consensus. While specialized collagen stains (e.g., Masson's trichrome) provide higher specificity for fibrosis, the characteristic stromal densification, hyalinization, and architectural changes were readily identifiable and consistently scored on H&E-stained sections in this study.

Statistical analysis was performed using SPSS software, version 28.0. The primary unit of analysis was the individual eye. This approach is justified by the strictly local nature of transscleral laser irradiation and the absence of systemic effects from the photosensitizer or the procedure itself, aligning with the ethical 3R principle of Reduction to minimize animal use. To account for the potential inter-eye correlation within the same animal, all statistical comparisons were performed using Generalized Estimating Equations (GEE) with an exchangeable correlation structure, treating eyes as nested within rabbits. For binary outcomes (presence of thrombosis, fibrosis, retinal or scleral damage), a logistic GEE model was used. For ordinal histological severity scores, an ordinal logistic GEE model was applied. Results are presented as adjusted odds ratios (OR) with 95% confidence intervals (CI). For continuous outcomes (temperature measurements), a linear mixed-effects model with rabbit ID as a random intercept was used. As a supplementary analysis, Fisher's exact test was used for comparison of proportions in 2x2 tables. All *P*-values are two-sided, and a value of < 0.05 was considered statistically significant.

Results

The results are presented at eye level. In the interpretation of the findings, the potential correlation of outcomes within the same animal was accounted for using GEE and mixed-effects models, as described in the methods.

As shown in Table 1, TS-PDT had a distinct impact on the sclera–choroid–retina complex, with the most pronounced changes in the choroid. GEE analysis, accounting for inter-animal clustering, revealed a significant association between chlorin e6 administration and choroidal vascular thrombosis (90% vs. 50%; adjusted OR = 9.8, 95% CI: 1.4 - 68.9; $P = 0.021$), highlighting the role of chlorin e6 in initiating microcirculatory disturbances (Figure 2). The severity of thrombosis was also significantly higher in the TS-PDT group (adjusted OR for a one-point increase in score = 5.2, 95% CI: 2.1 - 12.9; $P < 0.001$), confirming the development of more extensive vascular injury.

Histologically, thrombi appeared as dense eosinophilic masses, partially or fully obstructing vessel lumina, with aggregated erythrocytes and fibrin. Figure 2 shows a representative thrombus filling most of a large choroidal vessel.

The study demonstrated choroidal stromal fibrosis following laser exposure, with a significantly higher incidence in the photosensitizer group (80% vs. 40%; adjusted OR = 6.2, 95% CI: 1.1 - 35.0; $P = 0.039$), indicating the role of chlorin e6 in promoting collagen deposition and stromal remodeling (Figure 3). Fibrosis severity was also greater in the treated group (adjusted OR = 4.8, 95%

CI: 1.9 - 12.1; $P<0.001$), confirming the contribution of the photosensitizer to more extensive fibrotic changes. Histologically, collagen fibers in the TS-PDT group appeared thickened and hyalinized, with reduced cellularity and vascularity compared to the normal choroid. Figure 3 illustrates marked stromal thickening and densification. In the TSLT group, histological changes in the choroid were not only significantly less pronounced but also differed in character. One common finding was stromal edema. Figure 4 illustrates loosening of the choroidal stromal matrix, increased intercellular spaces, and mild perivascular infiltration. Vascular lumina remained largely unobstructed. Additionally, dilated (ectatic) vessels without signs of thrombosis or endothelial injury were frequently observed in this group. Figure 5 shows choroidal vessels with widened lumina and intact vessel walls, lacking evidence of thrombus formation or structural damage.

To assess perifocal tissue reactions adjacent to the "zone of interest,"a histological examination of the retina was performed. The analysis revealed a minimal number of eyes with pathological changes - limited to localized edema and small foci of necrosis - observed only once in each group: 10% (1/10 eyes) in both the photosensitizer-treated and control groups. The mean severity scores were also comparable: 0.5 ± 0.2 in the TS-PDT group and 0.6 ± 0.3 in the TSLT group. Statistical analysis using GEE revealed no significant differences in any of the parameters assessed ($P=1.00$), suggesting the preservation of retinal structural integrity regardless of photosensitizer use. A similar trend was observed in the histological analysis of the sclera.

Pathological changes, such as collagen fiber homogenization and focal sclerosis, were detected in 15% (1/10 eyes) of the TS-PDT group and 20% (2/10 eyes) of the TSLT group. The average severity scores were likewise comparable: 0.7 ± 0.3 and 0.8 ± 0.4, respectively. No statistically significant differences were found using GEE ($P=0.87$), confirming the short-term structural preservation of scleral tissue with this method.

It is important to emphasize that thermal parameters, including baseline temperature, temperature after 5 and

Table 1. Comparative Analysis of Tissue Complex Alterations in the Rabbit Eye ("Sclera–Choroid–Retina") Following Transscleral Photodynamic Therapy and Transscleral Laser Therapy without a Photosensitizer

Parameter	Group 1 (TS-PDT)	Group 2 (TSLT)	*P*-value (GEE/Mixed Model)	Supplementary *P*-value (Fisher's Exact Test)
Number of animals	5	5	-	-
Total number of eyes	10	10	-	-
Body weight (kg)	3,0–3,5	3,0–3,5	-	-
Baseline T (°C)	36,5 ± 0,7	36,8 ± 0,6	0,45	-
T at 5 min (°C)	39,5 ± 0,9	39,2 ± 0,8	0,67	-
T at 10 min (°C)	40,2 ± 1,0	40,5 ± 1,1	0,53	-
ΔT (°C)	3,7 ± 0,3	3,7 ± 0,4	0,89	-
Presence of choroidal vascular thrombosis (%)	90 (9/10)	50 (5/10)	0.021	0.165
Thrombosis severity (score)	3,2 ± 0,5	1,8 ± 0,6	<0,001	-
Presence of choroidal stromal fibrosis (%)	80 (8/10)	40 (4/10)	0.039	0.371
Stromal fibrosis severity (score)	2,8 ± 0,4	1,5 ± 0,5	<0,001	-
Retinal damage (% of affected eyes)	10 (1/10)	10 (1/10)	1,00	1,00
Retinal damage severity (score)	0,5 ± 0,2	0,6 ± 0,3	0,87	-
Scleral damage (% of affected eyes)	15 (1/10)	20 (2/10)	0,87	1.00
Scleral damage severity (score)	0,7 ± 0,3	0,8 ± 0,4	0,92	-

Note: P-values for binary and ordinal outcomes derived from Generalized Estimating Equations (GEE). P-values for temperature outcomes derived from linear mixed-effects models. Supplementary P-values for proportions from Fisher's exact test

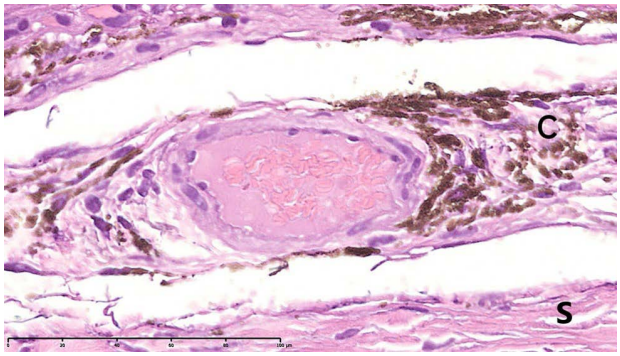

Figure 2. Transscleral photodynamic therapy with chlorin e6 (0.17 W). A representative thrombus (arrow) filling a large choroidal vessel. Hematoxylin and eosin (H&E) staining. Scale bar = 100 µm. S: sclera, C: choroid. × 700

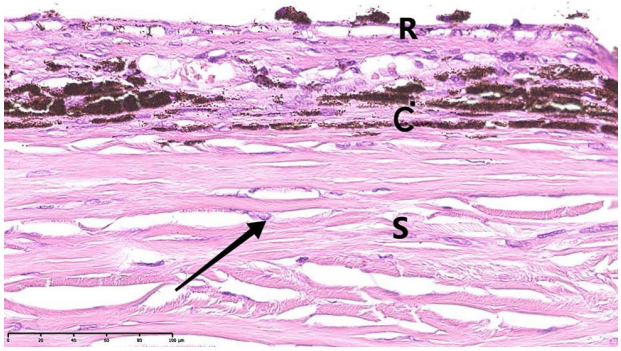

Figure 3. Transscleral photodynamic therapy with chlorin e6 (0.17 W). Choroidal stromal fibrosis (arrows). Hematoxylin and eosin (H&E) staining. Scale bar = 100 µm. S: sclera, C: choroid, R: retina. × 400.

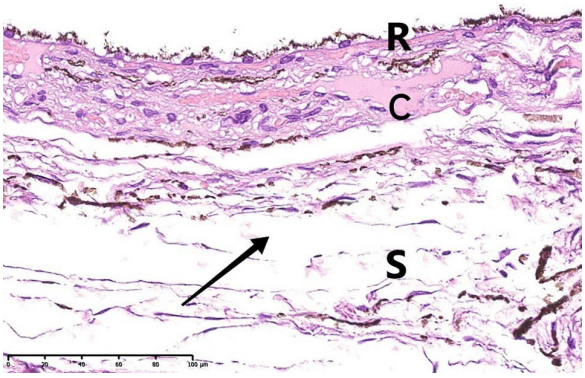

Figure 4. Transscleral laser therapy without a photosensitizer (0.17 W). Stromal edema in the choroid (arrows). Hematoxylin and eosin (H&E) staining. Scale bar = 100 µm. S: sclera, C: choroid, R: retina. × 400

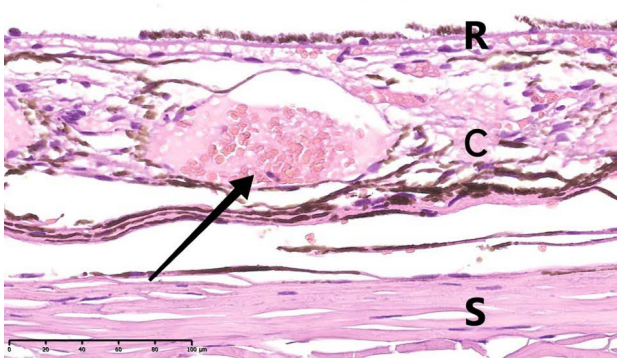

Figure 5. Transscleral laser therapy without a photosensitizer (0.17 W). Ectatic choroidal vessels (arrows). Hematoxylin and eosin (H&E) staining. Scale bar = 100 µm. S: sclera, C: choroid, R: retina. × 400

10 minutes, and ΔT, did not show statistically significant differences between the groups in the linear mixed-effects model ($P \geq 0.45$). This further supports the interpretation that the vascular effects observed in the TS-PDT group are primarily due to the action of the photosensitizer, with no significant contribution from differential surface heating detected under these conditions.

Discussion

This experimental study demonstrates that TS-PDT with chlorin e6 induces selective damage to the choroidal vasculature, clearly distinct from thermal injury produced by identical laser parameters without a photosensitizer. Chlorin e6 is one of the most thoroughly studied second-generation photosensitizers, widely applied in PDT for various diseases.[2,5,8,23,25-27,33,35] Its absorption peak (~660 nm) enables effective light penetration through the sclera and pigmented tissues.[5,21,22] Its preferential accumulation in vascular endothelium, confirmed in prior studies, underlies its use in ocular oncology.[2,5,8,23] Upon laser activation, chlorin e6 generates singlet oxygen ($^1O_2$) and other reactive oxygen species[11], initiating endothelial injury, platelet aggregation, and thrombus formation.[23] Additional advantages include high photochemical reactivity and low systemic toxicity.[2,5,26,29,33] Inclusion of a control group enabled differential analysis of photochemical versus thermal effects. This design - standard in PDT research - helps distinguish PS-driven responses from nonspecific changes due to heating or light absorption by pigmented tissues such as the retinal pigment epithelium.[12-20] The selected laser parameters (0.17 W, 0.866 W/cm², 519.5 J/cm²) were based on compensating for energy loss through the sclera and ensuring effective energy delivery to the choroid. Lower settings would risk inefficacy; higher settings could induce coagulative necrosis and suppress photochemical reactions.[31-34]

The control of laser exposure parameters, including accounting for power distribution inhomogeneity and the design features of transscleral probes (flat tips applied to the spherical surface of the eyeball), is a key aspect of minimizing complication risks and improving the reproducibility of TS-PDT. A power difference of approximately 20% between the center and edges of the exposure zone, although acceptable in clinical practice, requires careful planning of irradiation parameters. Along with this, variability in scleral thickness, pigmentation, and blood supply can also affect the uniformity and depth of energy distribution in tissues, leading to undesirable phenomena, especially in the context of thermal risks.[36,37,38] The depth of light attenuation, defined as the distance at which radiation intensity decreases to 37% of the original, depends on reflection, scattering, and absorption factors.[39,40] For the range of 625–640 nm, close to that used in this study (660 nm), the attenuation length is 3-5 mm, which correlates with the experimentally and clinically confirmed penetration depth of chlorin e6 and explains its advantage over first-generation drugs.[2,9,21] In addition, the high selectivity of chlorin e6 accumulation in pathological tissues and low systemic toxicity enhance the therapeutic profile of the method, minimizing the risks of damage to healthy structures.[2,8]

Significant differences were observed between the groups using GEE analysis; in the TS-PDT group, both the incidence and severity of thrombosis and fibrosis were markedly higher than in the controls. Importantly, no significant intergroup differences in thermal parameters ($P \geq 0.45$) were observed, confirming that no significant differences in monitored surface thermal elevation were detected under these exposure conditions. The vascular damage is attributable to photooxidative stress and downstream vasoactive mediator release (e.g., nitric oxide, thromboxane, calcium ions).[13,14,20]

In the control group, vascular alterations were milder and histologically different - mainly stromal edema and vascular ectasia - accompanied by low thrombosis and fibrosis rates. These features do not reflect a classical photodynamic pattern. Likewise, the absence of coagulative necrosis supports the non-thermal nature of the exposure. Conversely, thermal therapies typically rely on higher energy levels, producing temperature elevations in the range of 45–55 °C, which often result in coagulative necrosis - a phenomenon not observed in this study.[9,36] The laser parameters used in this experiment were substantially lower than those applied in thermotherapy, supporting the interpretation that, in the absence of a photosensitizer, the exposure represented an intermediate, subtherapeutic

mode incapable of inducing either a true photodynamic or significant thermal effect. Consistent monitored surface thermal parameters further confirm that vascular damage resulted from PS activation, with no significant contribution from differential surface heating.

These findings align with prior work showing that PDT at therapeutic power densities does not significantly raise tissue temperature.[16,17,19,20] For example, Grossman et al. reported a rise of just 0.3 °C during PDT - too low to affect biological tissues.[19] Our results are also in agreement with tumor model studies. In a CT26 colorectal carcinoma model, Orlova et al. (2022) showed that chlorin e6–based red-light PDT (660 nm) suppressed tumor oxygenation and perfusion, whereas light alone had no significant effect.[20] Similarly, Dolmans et al. reported only minimal, statistically insignificant changes from light exposure alone, in stark contrast to the robust effects observed with full PDT.[17]

Temperature control in this study was ensured by both appropriate energy selection and real-time thermal monitoring. The power density used (0.866 W/cm², recalculated for the sclera) remained within PDT's therapeutic window and below the threshold for irreversible thermal damage.[31-34] The monitoring system provided feedback that ensured the procedure stayed within photochemical limits. This is critical, as exceeding 42 °C is known to impair singlet oxygen generation and shift the mechanism toward thermal effects.[20,23,24,34,35]

Histologically, chlorin e6 initiated a cascade of endothelial damage, coagulation, and formation of fibrin--erythrocyte thrombi, followed by reparative fibrosis. GEE analysis confirmed the strong association of the photosensitizer with vascular pathology: odds of thrombosis were significantly higher (adjusted OR = 9.8; 95% CI: 1.4 - 68.9; $P = 0.021$), and fibrosis significantly higher (adjusted OR = 6.2; 95% CI: 1.1 - 35.0; $P = 0.039$), compared to the controls. These findings support the central role of chlorin e6 in triggering both key pathomorphological outcomes via photochemical mechanisms.

A major strength of the study is the confirmed short-term structural preservation of surrounding tissues with TS-PDT under the selected settings. No significant differences in retinal or scleral damage were observed between the groups; both remained minimal. This supports the principle of selectivity: photochemical injury is restricted to PS-accumulating structures (choroidal vessels), sparing the sclera (irradiation path) and retina (beyond the treatment zone). Importantly, scleral temperature remained below the destabilization threshold ($\Delta T = 3.7 \pm 0.3$ °C; peak 40.2 ± 1.0 °C), minimizing the risk of coagulative necrosis.[23,24,35]

To our knowledge, this is among the first ophthalmologic studies to assess the in vivo effects of TS-PDT using a controlled experimental model. This is crucial for clinical translation, as the eye's anatomy introduces complex variables: the sclera acts as a scattering and absorbing barrier, while deeper pigmented tissues (e.g., retinal pigment epithelium, intraocular tumors) can alter both energy absorption and heat distribution. The present data from healthy choroid models provide a baseline for future studies in tumor models, where variables such as pigmentation and neovascularization must be further investigated.

The main limitation of this study is the use of a healthy choroid model, which does not reflect tumor-specific features such as neovascularization, hypoxia, and heterogeneous melanin content. Nevertheless, the results confirm the photochemical nature and short-term structural preservation with TS-PDT and validate chlorin e6 as a photosensitizer - laying the groundwork for further preclinical and clinical research. Future directions include optimizing irradiation parameters, adapting treatment protocols to various tumor types, and exploring combinations with transpupillary PDT, thermotherapy, Ru-106/Rh-106 brachytherapy, and proton therapy.[30,41] Real-time thermal monitoring remains essential to prevent unintended thermal injury.

Conclusion

This study demonstrates that targeted vascular injury induced by transscleral laser exposure with chlorin e6 is mediated primarily by photochemical mechanisms. Comparative GEE analysis accounting for clustered data showed a significant increase in choroidal thrombosis and fibrosis when chlorin e6 was used, with no significant intergroup differences in monitored scleral surface temperatures - supporting the specificity of the photodynamic effect. Importantly, short-term structural preservation of retinal and scleral tissues was confirmed. These findings argue against the contribution of significant differential surface heating and provide strong experimental justification for the continued development of TS-PDT as an organ-preserving treatment for intraocular tumors.

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The authors declare no conflicts of interest.

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