Regular Article

Inhibition of Laser-Induced Choroidal Neovascularization by Hematoporphyrin Dimethylether-Mediated Photodynamic Therapy in Rats

Yi-Jia Yan,^{a,b} Lei-Lei Bao,^c Li-Jun Zhang,^a Jun Bian,^c Tai-shan Hu,^b Mei-zhen Zheng,^b Dan-Ye Chen,^a Xin-hai Yu,^{*,a} and Zhi-Long Chen^{*,a}

^aDepartment of Pharmaceutical Science & Technology, College of Chemistry and Biology, Donghua University; Shanghai 201620, China; ^bDepartment of Pharmacology, Shanghai Xianhui Pharmaceutical Co., Ltd.; Shanghai 200433, P. R. China; and ^c“411” Hospital; Shanghai 200081, P. R. China.

Received April 18, 2017; accepted September 15, 2017

This study aimed to investigate the effect of hematoporphyrin dimethylether (HDME)-mediated photodynamic therapy for laser-induced choroidal neovascularization (CNV) in adult Brown Norway rats. HDME was administered *via* tail vein at 14d after the laser photocoagulation, and the rats received irradiance with a laser light at 570 nm at 15 min after injection. CNV was evaluated by fundus photography, fundus fluorescein angiography, optical coherence tomography, and hematoxylin and eosin staining. We found that CNV was occurred at 7d after photocoagulation and reaching peak activity at 14d after photocoagulation. There is a significant reduction in the total area of the fluorescein leakage and the number of strong fluorescein leakage spots on 7d after HDME-mediated photodynamic therapy (PDT). The results suggest that HDME-mediated PDT inhibits laser-induced CNV in rats, representing a promising therapy for wet age-related macular degeneration.

Key words photodynamic therapy; hematoporphyrin dimethylether; choroidal neovascularization; age-related macular degeneration

Age-related macular degeneration (AMD) is a progressive, degenerative disease of the central retina causing irreversible blindness or loss of vision. AMD can be divided into two subtypes as wet (neovascular) and dry (atrophic), characterized by the presence of choroidal neovascularization (CNV).^{1,2} In wet AMD which is responsible for 80–90% of severe vision loss associated with AMD, CNV extends through Bruch's membrane into the sub-retinal space or sub-retinal pigment epithelium.^{3–5} The CNV can leak blood and fluid, and are accompanied by fibrous tissue, which often leads to damage of the retinal tissues and vision loss.⁶ The CNV is usually visible as a grayish green sub-retinal membrane, surrounded by a halo of pigmentation, sometimes with clearly visible sub-retinal blood, fluid, or lipids.⁷ At present, the removal of CNV is the key to the treatment of AMD. Conventional management of CNV includes laser photocoagulation,⁸ transpupillary thermotherapy (TTT),^{9,10} sub-macular surgical removal of CNV¹¹ and vascular endothelial growth factor (VEGF) inhibition.^{12,13} The efficacy of laser photocoagulation is not satisfactory. The Macular Photocoagulation Study Groups reported that the laser treatment of subfoveal CNV induced an immediate decrease in visual acuity, and they also showed that the neovascularization recurrence rate was rather high.^{14,15} The sub-macular surgical to remove CNV has great risk and surgical complications. VEGF trap and anti-VEGF antibodies for CNV are effective for maintaining and improving vision, however, a portion of patients do not respond to the treatment well.^{16–18} Moreover, current anti-VEGF drugs may increase the risk of complications such as the retinal pigment epithelium (RPE) tears,¹⁹ cardiovascular disease,²⁰ nervous system disease (transient ischemic attack)²¹ and other systemic adverse events.

Photodynamic therapy (PDT) has been introduced into

ophthalmology in the late 1990s as a treatment for CNV due to AMD.^{22,23} PDT is based on the use of photosensitizers that generate oxygen radicals when activated by light of the appropriate wavelength.²⁴ Since Verteporfin (Visudyne; Novartis AG, Basel, Switzerland) has been approved of U.S.A. Food and Drug Administration (FDA) for the treatment of CNV due to subfoveal lesions,^{25,26} PDT with Verteporfin has been shown to be effective and saved up to now more than a million eyes from legal blindness.^{27–31} Verteporfin is semisynthetic product derived from natural products hemin. It mainly absorbs light at 689 nm, which allows deeper tissue penetration (>1 mm) but may damage normal tissues such as the RPE

Fig. 1. UV-Vis Absorption Spectra for Hematoporphyrin Dimethylether (HDME) in DMSO at the Concentration of 10 μ M

The inserted figure is the chemical structure of HDME.

*To whom correspondence should be addressed. e-mail: yuxinhai@dhu.edu.cn; zlchen1967@qq.com

and photoreceptors.³²⁾ Therefore, the discovery and development of safe and effective photosensitizers for the treatment of AMD has attracted increasing attentions. It has been suggested that the laser light having a wavelength ranging from 430 to 600nm may be safer for PDT of CNV.³³⁾

Hematoporphyrin dimethylether (HDME) is a porphyrin-based photosensitizer, which exhibits five absorption bands from 300 to 650nm (shown in Fig. 1). Previous studies show that HDME is more effective for tumor *in vivo* than Photofrin II[®].^{34,35)} However, the efficacy and safety of HDME in treatment of CNV has not been reported. Herein, the experimental CNV in rats was induced by laser photocoagulation, and the effect of new photosensitizer HDME-mediated photodynamic therapy for laser-induced CNV in rats was evaluated by fundus color photography, fundus fluorescence angiography (FFA), optical coherence tomography (OCT) and histopathological analysis.

MATERIALS AND METHODS

Chemicals and Reagents Hematoporphyrin dimethylether (HDME) and verteporfin were supplied by Shanghai Xianhui Pharmaceutical Co., Ltd. All the chemicals and reagents were of analytical grade and used without any purification, and all the reagents were obtained from Sinopharm Chemical Reagent Co., Ltd.

Animals Seven week-old male Brown Norway (BN) pigmented rats were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. Their weights ranged from 170 to 210g. All animal protocols were approved by the Animal Care and Use Committee of Donghua University.

Laser-Induced CNV Eighteen BN rats were used to establish the experimental CNV by laser photocoagulation. The rats were anesthetized by intraperitoneal injection of pentobarbital (40mg/kg). Laser photocoagulation-induced rupture of Bruch's membrane was performed on the right eyes using the following laser parameters: 659nm, 100 μ m spot size, 120mW intensity, and 0.1s duration (VISULAS Trion, ZEISS, Germany). Six laser spots were applied between the major retinal vessels adjacent to the optic disc. Only laser lesion with a subretinal bubble indicating perforated Bruch's membrane was considered effective and thus included in the study.

Evaluation of Experimental CNV Sixteen BN rats were used to establish the experimental CNV by laser photocoagulation. On day 0, laser photocoagulation was performed as described above. The coagulated lesions of twelve rats ($n=72$) were assessed on days 1, 7, 14, 21 by means of ophthalmoscopy, fundus color photography, fluorescein angiography, OCT and another four rats were used for histopathological analysis on days 14.

Evaluation of the Effects of HDME-Mediated PDT on Experimental CNV Twelve male rats were randomly assigned into control group, HDME group and verteporfin group, each group includes four rats and underwent laser photocoagulation as described above. At 14d after the laser photocoagulation, the rats in PDT group were injected with HDME or verteporfin at a dose of 10mg/kg *via* the tail vein. PDT was performed at 15min after administration by a laser instrument for ophthalmology (Anywave Technology Co., Ltd., Beijing, China) on the eyes using the following laser parameters: 1800 μ m spot size, 600mW/cm², and 83s duration.

The wavelength of light used in HDME group is 570nm when that in verteporfin group is 689nm. The rats in control group were without any treatment.

Fundus Color Photography and FFA Fundus color photography and FFA were performed under systemic anesthesia and pupil dilation using a hand-held retinal camera (Genesis-Df, KOWA, Japan) and a 5.4mm contact fundus lens. For FFA, fluorescein sodium (100mg/mL, 0.5mL/kg) was injected

Fig. 2. Fundus Color Photography Taken before Photocoagulation, 7, 14 and 21 Days after Photocoagulation

The photocoagulation spots formed gray and white scars and the scars became deeper with the increase of time after photocoagulation from 7 to 14d.

Fig. 3. Fundus Fluorescence Angiography Taken before Photocoagulation, 7, 14 and 21 Days after Photocoagulation

Weak fluorescein staining was observed on day 7, the intensity increased on day 14, and the strong fluorescein staining was still present on day 21.

into the tail vein of anesthetized rats. Pictures were taken from 3 to 360s after injection. The laser lesions were studied using fluorescein angiography to evaluate CNV development and its activity.

OCT OCT scanning was performed using an OCT system, (Spectralis OCT, Heidelberg, Germany). Rats were positioned on a custom cassette that allowed aligning the mouse eye for imaging. Hydration with normal saline was used to preserve corneal clarity. Volume analysis centered on the optic nerve head was performed, using 100 horizontal, raster, and consecutive B-scan lines, each one composed by 2000 A-scans.

Histopathological Analysis Rats were given a lethal dose of sodium pentobarbital (50mg/kg intraperitoneally), and eyes were enucleated. The anterior segments were removed, and the posterior eyecups were post-fixed in 4% paraformaldehyde (PFA) for 2h and transferred to 30% sucrose/phosphate buffered saline (PBS) overnight. Then tissue sections at 6 μ m thickness were obtained from paraffin embedded tissue blocks. After washed in xylene to remove the paraffin, rehydrated with serial dilutions of ethanol, and washed in distilled water, sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope (Olympus, Japan).³⁶⁾

Evaluation and Statistical Analysis CNV formation

Fig. 4. The Intensity of FFA Leakage Grade (a.) and the Fluorescence Leakage Size (b.) at 1 Day, 7, 14 and 21 Days after Photocoagulation (** $p < 0.01$, significantly different from 1d after photocoagulation)

Fig. 5. OCT Images (a) and Light Microscopy (b) of Retinal Lesions with H&E ($\times 200$) from Control and 14 Days after Photocoagulation (GCL, Ganglion Cell Layer; INL, Inner Nuclear Layer; ONL, Outer Nuclear Layer)

and the effect of PDT treatment were evaluated by fundus color photography, FFA, OCT and histopathological analysis. The intensity of staining in late-phase (100–140s after dye injection) fluorescein angiography was graded as follows: no staining, Score 0; slightly stained, score 1; moderately stained, score 2; strongly stained, score 3.^{18,37)} The area of the CNV lesion was measured three times and averaged using Image pro-plus 6.0. Student's *t*-test was used to measure differences using the SPSS16.0 software package, and only *p*-values less than 0.05 were considered significant.

RESULTS

Fundus Color Photography Evaluation of Experimental CNV Figure 2 is the fundus color photography taken before photocoagulation, 7, 14, and 21 d after photocoagulation. It was showed that the photocoagulation spots formed gray and

white scars and the scars became deeper with the increase of time after photocoagulation from 7 to 14d. There was no significant change between 14 and 21 d after photocoagulation.

FFA Evaluation of Experimental CNV FFA is showed in Fig. 3, weak fluorescein staining was observed on day 7, the intensity increased on day 14, and the strong fluorescein staining was still present on day 21. The intensity of FFA leakage grade and the fluorescence leakage size at 1, 7, 14 and 21d after photocoagulation are displayed in Fig. 4. At 1d after photocoagulation, there was no spot showing strong staining (score 2 or more). At 7d after photocoagulation, most of the spots showed strong staining at score 2, the number of spot show strong staining at score 3 were increased with the increase of time until 14d after photocoagulation. A significant increase in fluorescence leakage size from 1 to 14d after photocoagulation was observed, and there was no significant change between 14 and 21 d after photocoagulation.

Fig. 6. Fundus Fluorescence Angiography

a. The intensity of FFA leakage grade b. The fluorescence leakage size of model group, HDME-PDT group and verteporfin-PDT group at 7 d after PDT (** $p < 0.01$, significantly different from model group) c. Fundus fluorescence angiography taken before and 7 d after HDME-PDT and verteporfin-PDT. At 7 d after PDT, some photocoagulation spots (gray arrow indication) showed weaker staining intensity in fundus fluorescence angiography than that before PDT.

OCT and Histopathological Analysis Evaluation of Experimental CNV The OCT image and light microscopy of retinal lesions with H&E (Fig. 5) showed that the layers of retina and choroid in control rats were distinct and the structures were integrated. At 14d after photocoagulation, we observed hyperreflectivity in the RPE and the outer photoreceptor region. The outer plexiform layer (OPL) folded toward the outer nuclear layer (ONL) forming an arch pattern and the hyper-

reflective subretinal lesion were differentiated easily from the surrounding retina (Fig. 5a). These findings correlated with the histological findings which revealed disruption and necrosis of the outer retina and RPE (Fig. 5b). Furthermore, a prominent subretinal fibrovascular complex was observed by H&E staining.

The Effects of HDME-Mediated PDT on Experimental CNV At 7d after HDME-PDT and verteporfin-PDT, the

Fig. 7. OCT Images of Retinal Lesions before and 7 Days after HDME-PDT and Verteporfin-PDT

At 7d after PDT, the integrity of retina and choroid was recovered in a certain extent. The arch pattern forming by outer plexiform layer (OPL) and outer nuclear layer (ONL) became smaller.

Fig. 8. CNV Size (a) and Light Microscopy (b) of Retinal Lesions with H&E (×200) from Model Group and Treatment Group at 7 Days after PDT

At 7d after PDT, the CNV size was significantly decreased compared with that in the model group.

score given to the staining in FFA were lower than model group and the fluorescence leakage size was smaller than that in model group (Fig. 6). The number of spots that showed strong dye staining (score 3) were decreased and the number showing weak staining (score 1 and score 0) increased at 7 d after HDME-PDT and verteporfin-PDT (Fig. 6a). The fluorescence leakage size of decreased by 51.14 and 68.37% compared with model group, respectively (Fig. 6b). As shown in Fig. 6c, at 7 d after HDME-PDT and verteporfin-PDT, some photocoagulation spots (gray arrow indication) showed weaker staining intensity in fundus fluorescence angiography than that before PDT. In verteporfin-PDT group, the retinal blood vessels were fuzzy and the whole fundus appeared weak fluorescence leakage compared with HDME-PDT group.

Figure 7 is OCT images of retinal lesions before and 7 d after HDME-PDT. At 7 d after PDT, the integrity of retina and choroid was recovered in a certain extent. The results showed that the arch pattern forming by outer plexiform layer (OPL) and outer nuclear layer (ONL) all became smaller in visudyne-PDT and HDME-PDT groups. After 7 d, the arch pattern size was decreased by 41.67% in HDME-PDT group, especially. This corresponded to a significantly decrease of CNV size seen on H&E staining compared with model group (Fig. 8). The CNV size of HDME-PDT group and verteporfin-PDT group were decreased by 48.79 and 67.56% compared with model group, respectively.

DISCUSSION

CNV is the hallmark of wet AMD and is responsible for most vision loss attributed to the AMD.³⁸⁾ Hence, a new therapy for CNV in AMD has been long-awaited. The development of the laser-induced CNV model, which has had a significant impact on our understanding of the mechanism and treatment of CNV, has become the most widely used model in wet AMD research.³⁹⁾ Since many animal models, including monkey,^{39,40)} rabbits,⁴¹⁾ cats⁴²⁾ and rats,^{43,44)} have been developed and used to evaluate the effect of various antiangiogenic agents on CNV. Among the numerous animal models, the pigmented rats have been used by several groups because its retina is similar to the human retina and is homogeneously pigmented and exhibited a high incidence of CNV after photocoagulation. Herein, we establish the experimental CNV in the subretinal space of BN pigmented rats by laser photocoagulation.

The experimental CNV were confirmed by fundus color photography, FFA, OCT and histopathological analysis. Similar to previous reports,^{45–47)} the results of fundus color photography, FFA, OCT and histopathological analysis with H&E confirmed the presence of CNV at 14 d after photocoagulation. And the CNV remained at a high level at 21 d after photocoagulation. On the basis of the time course of laser-induced CNV, we opted to perform HDME-PDT at 14 d after photocoagulation.

PDT has been shown to be effective in closing both experimental CNV in animal models as well as subfoveal CNV in humans.^{48,49)} PDT is an approved therapeutic procedure to treat cancers using combinations of chemical photosensitizers and light.²⁴⁾ Currently, photosensitizers most commonly used clinically or in clinical trials are porphyrin derivatives.⁵⁰⁾ Porphyrins are a class of naturally occurring compounds which have become intensively studied in last decades due to their

unique photochemical properties.⁵¹⁾ HDME is a porphyrin-based photosensitizer.

In the present study, we found that HDME-mediated PDT and verteporfin-mediated PDT results in a significant reduction of the total area of the fluorescein leakage and the number of strong fluorescein leakage spots. Although the effect of HDME was lower than verteporfin, the drug safety was higher. So the HDME had an ability which could be used for retreatment of CNV. In addition, the retinal blood vessels of HDME-PDT group was more clear than verteporfin-PDT group. The results suggest that HDME-mediated PDT is a promising therapy for CNV due to wet AMD.

Acknowledgments This work was supported by National Natural Science Foundation of China (Grant No. 21402236, 21372042), Foundation of Science and Technology Commission of Shanghai Municipality (No. 14431906200, 15431904100, 15XD1523400, 14ZR1439800, 14ZR1439900, 15ZR1439900), Fundamental Research Funds for the Central Universities (17D110513), Foundation of Science and Technology Commission of Songjiang District (16SJGG20).

Conflict of Interest The authors declare no conflict of interest.

REFERENCES

- 1) Cruess A, Maberley D, Wong D, Chen J. The treatment of wet AMD in Canada: access to therapy (policy review). *Can. J. Ophthalmol.*, **44**, 548–556 (2009).
- 2) Barak Y, Heroman WJ, Tezel TH. The past, present, and future of exudative age-related macular degeneration treatment. *Middle East Afr. J. Ophthalmol.*, **19**, 43–51 (2012).
- 3) Ferreira A, Sakriotis A, Olson M, Lu J, Makin C, Milnes F. Treatment frequency and dosing interval of ranibizumab and aflibercept for neovascular age-related macular degeneration in routine clinical practice in the USA. *PLOS ONE*, **10**, e0133968 (2015).
- 4) Calabrèse A, Bernard JB, Hoffart L, Faure G, Barouch F, Conrath J, Castet E. Wet versus dry age-related macular degeneration in patients with central field loss: different effects on maximum reading speed. *Invest. Ophthalmol. Vis. Sci.*, **52**, 2417–2424 (2011).
- 5) Seo MS, Kwak N, Ozaki H, Yamada H, Okamoto N, Yamada E, Fabbro D, Hofmann F, Wood JM, Campochiaro PA. Dramatic inhibition of retinal and choroidal neovascularization by oral administration of a kinase inhibitor. *Am. J. Pathol.*, **154**, 1743–1753 (1999).
- 6) Kent D, Sheridan C. Choroidal neovascularization: a wound healing perspective. *Mol. Vis.*, **9**, 747–755 (2003).
- 7) Miller DG, Singerman LJ. Vision loss in younger patients: a review of choroidal neovascularization. *Optom. Vis. Sci.*, **83**, 316–325 (2006).
- 8) Macular Photocoagulation Study Group. Laser photocoagulation of subfoveal neovascular lesions in age-related macular degeneration. Results of a randomized clinical trial. *Arch. Ophthalmol.*, **109**, 1220–1231 (1991).
- 9) Rogers AH, Reichel E. Transpupillary thermotherapy of subfoveal occult choroidal neovascularization. *Curr. Opin. Ophthalmol.*, **12**, 212–215 (2001).
- 10) Reichel E, Berrocal AM, Ip M, Kroll AJ, Desai V, Duker JS, Puliafito CA. Transpupillary thermotherapy of occult subfoveal choroidal neovascularization in patients with age-related macular degeneration. *Ophthalmology*, **106**, 1908–1914 (1999).
- 11) Lewis H, Medendorp SVB. Tissue plasminogen activator-assisted surgical excision of subfoveal choroidal neovascularization in age-related macular degeneration: a randomized, double-masked trial.

- Ophthalmology*, **104**, 1847–1851, discussion, 1852 (1997).
- 12) Singh B, Narayanan R. Re: Ranibizumab monotherapy versus single-session verteporfin photodynamic therapy combined with as-needed ranibizumab treatment for the management of neovascular age-related macular degeneration. *Retina*, **32**, 206, author reply, 206–207 (2012).
 - 13) Bashshur ZF, Schakal AR, El-Mollayess GM, Arafat S, Jaafar D, Salti HI. Ranibizumab monotherapy versus single-session verteporfin photodynamic therapy combined with as-needed ranibizumab treatment for the management of neovascular age-related macular degeneration. *Retina*, **31**, 636–644 (2011).
 - 14) Macular Photocoagulation Study Group. Persistent and recurrent neovascularization after krypton laser photocoagulation for neovascular lesions of age-related macular degeneration. *Arch. Ophthalmol.*, **108**, 825–831 (1990).
 - 15) Macular Photocoagulation Study Group. Persistent and recurrent neovascularization after krypton laser photocoagulation for neovascular lesions of ocular histoplasmosis. *Arch. Ophthalmol.*, **107**, 344–352 (1989).
 - 16) Bakall B, Folk JC, Boldt HC, Sohn EH, Stone EM, Russell SR, Mahajan VB. Aflibercept therapy for exudative age-related macular degeneration resistant to bevacizumab and ranibizumab. *Am. J. Ophthalmol.*, **156**, 15–22.e1 (2013).
 - 17) Wickremasinghe SS, Xie J, Lim J, Chauhan DS, Robman L, Richardson AJ, Hageman G, Baird PN, Guymer R. Variants in the APOE gene are associated with improved outcome after anti-VEGF treatment for neovascular AMD. *Invest. Ophthalmol. Vis. Sci.*, **52**, 4072–4079 (2011).
 - 18) Wang L, Yang ZK, Yu Y, Cui C, Guan HJ, Chen H. Blockage of tissue factor ameliorates the lesion of laser-induced choroidal neovascularization in mice. *Exp. Eye Res.*, **127**, 117–123 (2014).
 - 19) Arias L, Caminal JM, Rubio M, Pujol O, Arruga J. Retinal pigment epithelial tears after intravitreal bevacizumab injection for predominantly classic choroidal neovascularization. *Eur. J. Ophthalmol.*, **17**, 992–995 (2007).
 - 20) Valmaggia C, Niederberger H, Lang C, Kloos P, Haueter I. The treatment of choroidal neovascularisation with intravitreal injections of bevacizumab (Avastin). *Klin. Monatsbl. Augenheilkd.*, **225**, 380–384 (2008).
 - 21) Spaide RF, Chang LK, Klancnik JM, Yannuzzi LA, Sorenson J, Slakter JS, Freund KB, Klein R. Prospective study of intravitreal ranibizumab as a treatment for decreased visual acuity secondary to central retinal vein occlusion. *Am. J. Ophthalmol.*, **147**, 298–306 (2009).
 - 22) Schmidt-Erfurth U, Miller J, Sickenberg M, Bunse A, Laqua H, Gragoudas E, Zografos L, Birngruber R, van den Bergh H, Strong A, Manjurijs U, Fsadni M, Lane AM, Piguet B, Bressler NM. Photodynamic therapy of subfoveal choroidal neovascularization: clinical and angiographic examples. *Graefes Arch. Clin. Exp. Ophthalmol.*, **236**, 365–374 (1998).
 - 23) van den Bergh H. Photodynamic therapy of age-related macular degeneration: History and principles. *Semin. Ophthalmol.*, **16**, 181–200 (2001).
 - 24) Allison RR, Moghissi K. Photodynamic Therapy (PDT): PDT Mechanisms. *Clin. Endosc.*, **46**, 24–29 (2013).
 - 25) Tzekov R, Lin T, Zhang KM, Jackson B, Oyejide A, Orilla W, Kulkarni AD, Kuppermann BD, Wheeler L, Burke J. Ocular changes after photodynamic therapy. *Invest. Ophthalmol. Vis. Sci.*, **47**, 377–385 (2006).
 - 26) Singh SP, Sharma M, Patel H, Gupta PK. Extra cellular pH influences uptake and photodynamic action of pyropheophorbide—a entrapped in folate receptor targeted organically modified silica nanoparticle. *Photodiagnosis Photodyn. Ther.*, **11**, 156–164 (2014).
 - 27) Nowak-Sliwinska P, van den Bergh H, Sickenberg M, Koh AHC. Photodynamic therapy for polypoidal choroidal vasculopathy. *Prog. Retin. Eye Res.*, **37**, 182–199 (2013).
 - 28) Costagliola C, Campa C, Incorvaia C, Parmeggiani F, Menzione M, Della Corte M, Rinaldi M, Romano M, Semeraro F. Verteporfin photodynamic therapy for subfoveal choroidal neovascularization in pathologic myopia: a 12-month retrospective review. *Eur. J. Ophthalmol.*, **18**, 955–959 (2008).
 - 29) Hussain N, Khanna R, Das T, Narayanan R, Sunday OT, Bansal AG, Reddy R. Two years follow-up outcome of verteporfin therapy for subfoveal choroidal neovascularization in pathologic myopia in Indian eyes. *Indian J. Ophthalmol.*, **56**, 465–468 (2008).
 - 30) Blinder KJ, Blumenkranz MS, Bressler NM, Bressler SB, Donato G, Lewis H, Lim JI, Menchini U, Miller JW, Mones JM, Potter MJ, Pournaras C, Reaves A, Rosenfeld P, Schachat AP, Schmidt-Erfurth U, Sickenberg M, Singerman LJ, Slakter JS, Strong HA, Virgili G, Williams GA. Verteporfin therapy of subfoveal choroidal neovascularization in pathologic myopia: 2-year results of a randomized clinical trial—VIP report no. 3. *Ophthalmology*, **110**, 667–673 (2003).
 - 31) Verteporfin in Photodynamic Therapy Study Group. Photodynamic therapy of subfoveal choroidal neovascularization in pathologic myopia with verteporfin. 1-year results of a randomized clinical trial—VIP report no. 1. *Ophthalmology*, **108**, 841–852 (2001).
 - 32) Li T, Hou X, Deng H, Zhao J, Huang N, Zeng J, Chen H, Gu Y. Liposomal hypocrellin B as a potential photosensitizer for age-related macular degeneration: pharmacokinetics, photodynamic efficacy, and skin phototoxicity *in vivo*. *Photochem. Photobiol. Sci.*, **14**, 972–981 (2015).
 - 33) Deng H, Li TH, Xie J, Huang NY, Gu Y, Zhao JQ. Synthesis and bio-evaluation of novel hypocrellin derivatives: potential photosensitizers for photodynamic therapy of age-related macular degeneration. *Dyes Pigm.*, **99**, 930–939 (2013).
 - 34) Luksiene Z, Juzenas P, Moan J. Radiosensitization of tumours by porphyrins. *Cancer Lett.*, **235**, 40–47 (2006).
 - 35) Luksiene Z, Labeikyte D, Juodka B, Moan J. Mechanism of radiosensitization by porphyrins. *J. Environ. Pathol. Toxicol. Oncol.*, **25**, 293–306 (2006).
 - 36) Li F, Zeng Y, Xu H, Yin ZQ. Subretinal transplantation of retinal pigment epithelium overexpressing fibulin-5 inhibits laser-induced choroidal neovascularization in rats. *Exp. Eye Res.*, **136**, 78–85 (2015).
 - 37) Takehana Y, Kurokawa T, Kitamura T, Tsukahara Y, Akahane S, Kitazawa M, Yoshimura N. Suppression of laser-induced choroidal neovascularization by oral tranilast in the rat. *Invest. Ophthalmol. Vis. Sci.*, **40**, 459–466 (1999).
 - 38) Giani A, Thanos A, Roh MI, Connolly E, Trichonas G, Kim I, Gragoudas E, Vavvas D, Miller JW. *In vivo* evaluation of laser-induced choroidal neovascularization using spectral-domain optical coherence tomography. *Invest. Ophthalmol. Vis. Sci.*, **52**, 3880–3887 (2011).
 - 39) Ryan SJ. The development of an experimental model of subretinal neovascularization in disciform macular degeneration. *Trans. Am. Ophthalmol. Soc.*, **77**, 707–745 (1979).
 - 40) Ohkuma H, Ryan SJ. Experimental subretinal neovascularization in the monkey. Permeability of new vessels. *Arch. Ophthalmol.*, **101**, 1102–1110 (1983).
 - 41) van der Zypen E, Fankhauser F, Raess K, England C. Morphologic findings in the rabbit retina following irradiation with the free-running neodymium-YAG laser. Disruption of Bruch's membrane and its effect on the scarring process in the retina and choroid. *Arch. Ophthalmol.*, **104**, 1070–1077 (1986).
 - 42) Perry DD, Risco JM. Choroidal microvascular repair after argon laser photocoagulation. *Am. J. Ophthalmol.*, **93**, 787–793 (1982).
 - 43) Pollack A, Heriot WJ, Henkind P. Cellular processes causing defects in Bruch's membrane following krypton laser photocoagulation. *Ophthalmology*, **93**, 1113–1119 (1986).
 - 44) Baurmann H, Sasaki K, Chioralia G. Investigations on laser coagulated rat eyes by fluorescence angiography and microscopy. *Albrecht Von Graefes Arch. Klin. Exp. Ophthalmol.*, **193**, 245–252

- (1975).
- 45) Vásquez López G, Jaurrieta Alegria M, Munuera JM, Aliseda Pérez De Madrid D, Moreno Montañés J, Maldonado López M, García-Layana A. [Experimental model of subretinal neovascularization]. *Arch. Soc. Esp. Oftalmol.*, **75**, 675–680 (2000).
- 46) Cui J, Liu Y, Zhang J, Yan H. An experimental study on choroidal neovascularization induced by Krypton laser in rat model. *Photomed. Laser Surg.*, **32**, 30–36 (2014).
- 47) Zhao SH, He SZ. Study on the experimental model of krypton laser-induced choroidal neovascularization in the rats. *Zhonghua Yan Ke Za Zhi*, **39**, 298–302 (2003).
- 48) Woodburn KW, Engelman CJ, Blumenkranz MS. Photodynamic therapy for choroidal neovascularization: a review. *Retina*, **22**, 391–405, quiz, 527–528 (2002).
- 49) Incorvaia C, Campa C, Parmeggiani F, Menzione M, D'Angelo S, Corte MD, Rinaldi M, Romano M, Dell'Omo R, Costagliola C. 12-month retrospective study and review of photodynamic therapy with verteporfin for subfoveal choroidal neovascularization in age-related macular degeneration. *Retina*, **28**, 289–297 (2008).
- 50) Master A, Livingston M, Sen Gupta A. Photodynamic nanomedicine in the treatment of solid tumors: perspectives and challenges. *J. Control. Release*, **168**, 88–102 (2013).
- 51) Tomanová P, Rimpelova S, Jurasek M, Budesinsky M, Vejvodova L, Ruml T, Kmonickova E, Drasar PB. Trilobolide-porphyrin conjugates: On synthesis and biological effects evaluation. *Steroids*, **97**, 8–12 (2015).