Investigating retinal toxicity of a lutein-based dye in a model of isolated and perfused bovine retina

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Abstract

Purpose Retidyne™ is a new lutein-based dye for internal limiting membrane staining. It uses the intrinsic staining characteristics of lutein which is already known to act as an antioxidant and blue-light filter in the human retina. We investigated retinal tolerance to different staining times measured by the electroretinogram (ERG) of an isolated and perfused retina whole mount.
Methods For functionality, testing bovine retinas were prepared and perfused with an oxygen saturated standard solution and the ERG was recorded until stable b-wave amplitudes were reached. Then the perfusion was stopped and Retidyne™ was applied directly onto the retinal surface for exposure times of 60 or 120 s. After restarting the perfusion with standard solution, the ERG amplitudes were monitored for 75 min. To investigate the effects on photoreceptor function alone, 1 mM asparate was added to block b-waves.
Results For an exposure time of 60 s amplitudes of a- and b-waves remained stable throughout the experiment. Exposure times of 120 s caused an initial drop of amplitudes that reached statistical significance only for a-waves (a, -21%, $p = 0.047$; b, -14%, $p = 0.052$). This effect was only seen during the first minutes of the washout and the ERG recovered completely.
Conclusions In the model of isolated and perfused bovine retina, Retidyne™ showed a good safety profile for common intraoperatively used staining times. An initial toxic effect regarding the transient drop of amplitudes cannot be ruled out but the effect might also be explained by the partial blockage of the flashlight due to a more intense staining effect at the beginning of the washout.

Keywords Lutein · Vital dye · Internal limiting membrane staining · Peeling surgery · Electroretinogram · Biocompatibility

Introduction

In vitreoretinal surgery, dyes can be used to stain semi-transparent structures, e.g., when peeling the inner limiting membrane (ILM) or epiretinal membranes. This facilitates complete removal and may therefore improve the outcome and decrease the number of recurrences [1–3]. The ideal dye should provide selective staining of the target tissue, optimal contrast, and good biocompatibility. In the past, indocyanine green was the first choice when staining of the ILM was necessary. It had been used in ophthalmology for decades in the context of angiographies and was therefore considered as safe. Soon after its introduction in what is today called chromovitrectomy, doubts emerged regarding its safety as an intraocular dye. Alongside, visual-field defects, atrophies of the retinal pigment epithelium and the optic disc, reduced color vision, reductions in the electroretinogram, and a strong light-induced toxicity were observed [4–10]. ICG had not been tested specifically for an epiretinal application before.

A lutein-based dye seems promising regarding the abovementioned ideal properties.

Lutein alone has an orange color. Retidyne™ (Kemin Industries, Inc. Des Moines, IA USA 50317) contains 2% soluble lutein and 0.05% Brilliant blue. In this mixture, it stains the ILM with a green color and therefore enhances contrast. Because of its density it descents to the posterior pole without the need for fluid-air exchange.

Lutein is already approved and used as food colorant in the European Union as well as additive in animal feedstuff. Together with its structural isomer zeaxanthin, it belongs to

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the xanthophylls, a group of oxygen-containing carotenoids. Lutein and zeaxanthin (L/Z) were identified as the macular pigment in 1985 and play an important role in protection of the membranes of Henle fibers, which contain bundles of unmyelinated cone and rod photoreceptor axons, against photosensitized reactions [11]. They are delivered to the retina via plasma lipoproteins. Especially kale and spinach are rich in lutein and corn is an important source of zeaxanthin. In the absence of dietary modification or supplementation, the optical density of macular pigment is relatively stable [12]. Liu et al. [13] recently conducted a meta-analysis of eight randomized controlled trials which were considered as high quality and found a significant improvement in visual acuity and contrast sensitivity in patients with an age-related macular degeneration (AMD) after supplementation of xanthophyll carotenoids. Furthermore, there was a linear association between macular pigment optical density and the improvement of visual acuity and contrast sensitivity. There are animal studies showing that lutein and zeaxanthin could enhance lysosomal stability and reduce lipofuscin accumulation and so may reduce the risk of AMD [14, 15].

In conjunction with its intrinsic staining characteristics, these potentially neuroprotective properties and its role as a blue-light filter could provide retinal protection against increased oxidative stress and illumination during surgery [16, 17].

Therefore, we decided to investigate retinal tolerance to staining times of 60 and 120 s of the lutein-based dye Retidyne™ measured by the electroretinogram (ERG) of an isolated and perfused bovine retina, a sensitive tool for pharmacological research on retinal function [18, 19].

Material and methods

Aspartate, glucose, and other chemicals were obtained from Merck (Merck Pharma GmbH, Darmstadt, Germany) at pro analysis grade. Retidyne™ was donated by Peschke (Peschke Medizintechnik GmbH, Waldshut-Tiengen, Germany). The nutrient solution contained 120 mM NaCl, 2 mM KCl, 0.1 mM $MgCl_2$, 0.15 mM $CaCl_2$, 1.5 mM NaH_2PO_4, 13.5 mM Na_2HPO_4, and 5 mM glucose. To investigate photoreceptor function alone, 1 mM aspartate was added.

The electroretinogram (ERG) was recorded in the surrounding nutrient medium via two silver/silver-chloride electrodes on either side of the retina. Perfusion velocity was controlled by a roller pump and set to 1 ml/min. Temperature was kept constant at 30 °C. The perfusing medium was pre-equilibrated and saturated with oxygen. Retinas were dark-adapted and the ERG was elicited at intervals of 5 min using a single white xenon flash for stimulation. The flash intensity was set to 6.3 mlx at the retinal surface using calibrated neutral density filters (Kodak Wratten filter).

The duration of light stimulation was 10 μs controlled by a timer (Photopic Stimulator PS33 Plus; Grass, Warwick, RI). The ERG was filtered and amplified (100-Hz high filter, 50-hz notch filter, 100,000 × amplification) using a Grass RPS312RM amplifier. Data were processed and converted with an analog-to-digital data acquisition board (PCI-MIO-16XE-50; National Instruments, Austin, TX) in a desktop computer (PC compatible).

Each retina was perfused with the serum-free nutrient solution and stimulated repeatedly until stable b-wave amplitudes were recorded (Fig. 1 a) for at least 30 min. Thereafter, the perfusion was stopped and retinas were exposed to the dye for 60 or 120 s respectively. For all tests, retinas were reperfused with standard nutrient solution for 75 min and the changes of the b-wave amplitude were recorded. The b-
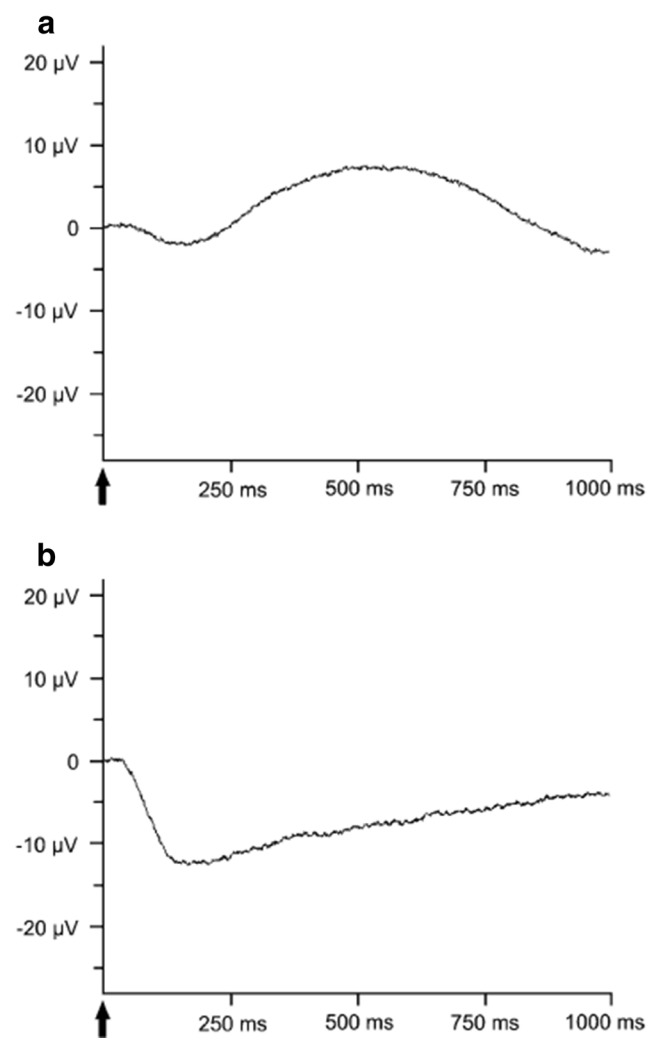

Fig. 1 The b-wave is dominant in the ERG of the isolated perfused bovine retina under scotopic light conditions. It results from a 10 ms light stimulus at a light intensity of 6.3 mlx (**a**). The a-wave is dominant in the ERG of the isolated perfused bovine retina after blocking the b-wave by adding 1 mM aspartate to the nutrient solution. The a-wave was generated by using a 10 ms light stimulus of 6.3 mlx at scotopic light conditions (**b**)

wave amplitude was measured from the trough of the a-wave to the peak of the b-wave.

To investigate the effect on the photoreceptor potential under scotopic conditions with a flash-intensity of 6.3 mlx, the b-wave was suppressed by adding 1 mM aspartate to the nutrient solution (Fig. 1 b). Aspartate is an inhibitor of synaptic transmission at the level of the first retinal synapse thus unmasking the photoreceptor potential by abolishing the b-wave. Under these conditions, we were able to investigate the effect of different staining times of the dyes on the photoreceptors.

After recording a stable photoreceptor potential for at least 30 min, we proceeded as described above using the same exposure times. The changes of the a-wave amplitude were recorded; recovery was followed up for 75 min. We calculated the percentage reduction of the a- and b-wave amplitudes for each exposure time.

The number of tested retina was five for each exposure time and for a- and b-wave measurements respectively.

Data analysis

Normal distribution was ensured for all data. The change of the ERG-amplitude 5 min after exposure to the dye and at the end of the reperfusion period of 75 min was compared with the last measured amplitude before exposure and the percentage was calculated for each time.

For statistical analysis, data was calculated throughout as the mean ± standard error of the mean. Significance was estimated by the Student's paired t test, $p \leq 0.05$ was considered to indicate a statistically significant difference.

Results

Stable ERG-amplitudes were obtained approximately 2 h after perfusion of the retinal whole mounts with standard solution. Environmental parameters such as pH, osmotic pressure, temperature, and pO_2 remained unchanged during all tests. When stable b-wave amplitudes were obtained, the perfusion was stopped and Retidyne™ was applied directly onto the retinal surface for exposure times of 60 or 120 s (Fig. 2 a, b). After restarting the perfusion with standard solution, the ERG amplitudes were monitored for 75 min. To investigate the effects on photoreceptor function alone, 1 mM asparate was added to block b-waves and we proceeded as with the recording of the b-wave amplitudes (Fig. 3 a, b).

Regarding a-waves, for an exposure time of 60 s there was a non-significant reduction of − 3.9% ($p = 0.37$) 5 min after restarting the perfusion and no difference at the end of the washout. For 120 s exposure time, a-wave amplitudes initially dropped significantly (− 21.0%, $p = 0.047$) and quickly recovered to + 2.0% after 75 min ($p = 0.37$).
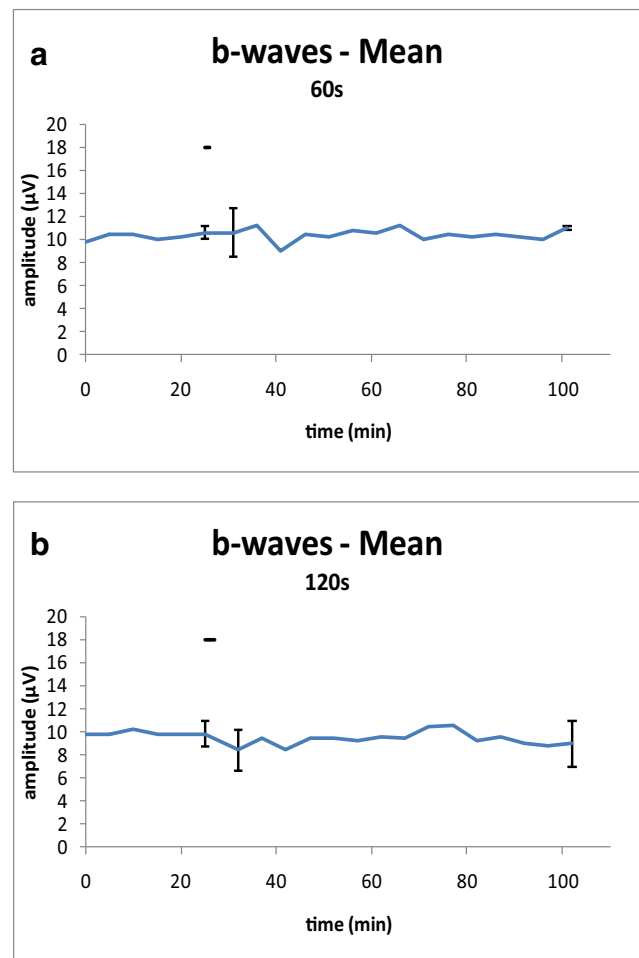

Fig. 2 Effects on the b-wave amplitude of the ERG of the isolated perfused bovine retina. Exposure times: 60s (**a**) and 120 s (**b**). Average of representative drug series (a, b; $n = 5$). The horizontal bar above the curve marks the exposition time. Three representative standard deviations for each drug series are given

For b-waves, an exposure time of 60 s did not cause any initial change, after 75 min of monitoring, there was an increase of + 3.8% which was not significant ($p = 0,18$). Exposing the retina to the dye for 120 s, we could observe an initial decrease of amplitudes similar to the a-waves, but statistical significance was not reached (− 14.0%, $p = 0.052$). At the end of the washout, a non-significant reduction remained (− 8.2%, $p = 0.37$).

Discussion

Today, there are several vital dyes competing against each other in terms of contrast enhancement, biocompatibility, and operative handling.

In the beginning of chromovitrectomy, the use of ICG was widespread. The application of this dye to enhance visibility of the ILM was first described by Kadonosono et al [20].
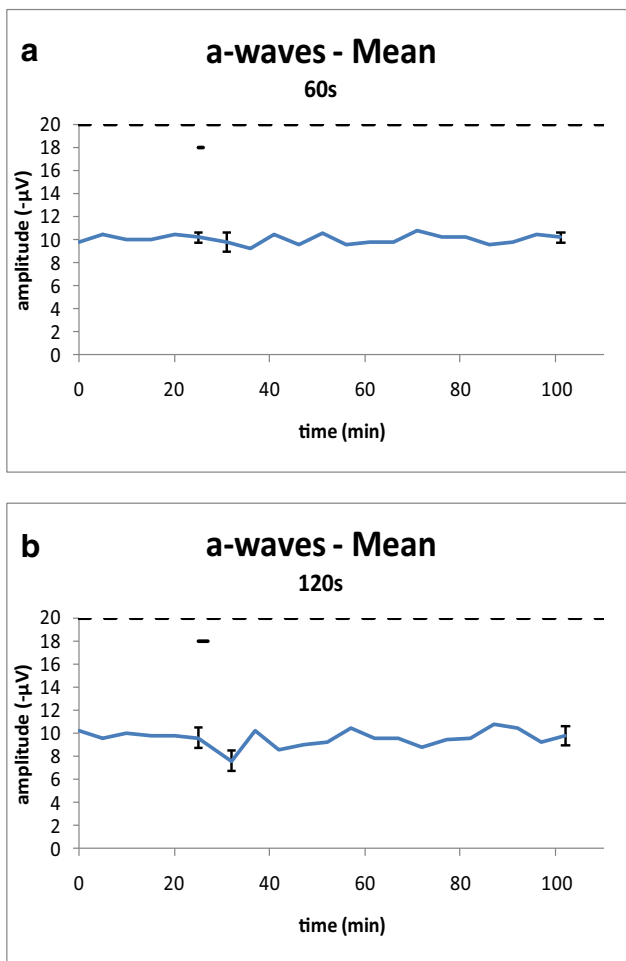
a a-waves - Mean
60s

b a-waves - Mean
120s

Fig. 3 Effects on the a-wave amplitude of the ERG of the isolated perfused bovine retina. Exposure times: 60s (**a**) and 120 s (**b**). Average of representative drug series (a, b; $n = 5$). The horizontal bar above the curve marks the exposition time. The dotted line marks the addition of aspartate [1 mM] to unmask photoreceptor potential. Three representative standard deviations for each drug series are given

Despite its assumed advantages like contrast enhancement and increasing the biomechanical stiffness of the ILM [21], the functional outcome of eyes after ILM peeling without ICG-staining turned out to be much better [22, 23]. As already mentioned above, visual-field defects, atrophies of the retinal pigment epithelium and the optic disc, reduced color vision, reductions in the electroretinogram, and a strong light-induced toxicity were observed in conjunction with the use of ICG [4–10]. On the basis of this experience, we learned that a new dye or a new application of an already existing dye necessitates a multimodal preclinical safety testing. It should be mentioned in this context that dyes are classified as medical devices and therefore do not need such an extensive safety testing during the approval procedure like for example a new drug. The legally required general cytotoxicity tests do not seem sufficiently adapted to a vitreoretinal application where the dye is in direct contact to sensitive retinal tissue [24].

A current example demonstrates the ongoing need for thorough preclinical in vitro and in vivo testing concerning vital dyes. Recently, Acid violet 17 (AV17) received certification for the European market as a dye for intraocular use during vitreoretinal interventions. This triarylmethane dye was preclinically tested in vivo after intravitreal injection in rabbit eyes. These eyes were not vitrectomized before injection. No signs of toxicity occurred in the ERG measurements and pathohistologic evaluations for concentrations of 0.25 mg/ml and 0.5 mg/ml. However, Tura et al. [25] tested the dye using the same experimental setup as we do as well as live/dead staining, immunohistochemistry, and immunoblotting and concluded that the application of AV17 appeared safe at a concentration of only 0.125 mg/ml or below. The actual dye concentration in the product entering the market was 1.5 mg/ml (ala purple, ala®-medics, Dornstadt/Germany). Following the clinical use of this formulation, there were several reports of severe central retinal toxicity which could be attributed to atrophy of the retinal pigment epithelium and damage on photoreceptor level. A recent review gives an overview on toxicity of AV17 which meanwhile is confirmed by different studies [26–28]. As a consequence, the dye was withdrawn from the market.

In our experimental model, ERG is derived immediately after exposure to the dye. The way of epiretinal application resembles the surgical procedure very well and we were able to define an exact time of contact between dye and retina. It is warranted that the actual concentration of a particular test solution is in direct contact with the retinal tissue, as the solution is not diluted by injecting it in a non-vitrectomized eye. The model of isolated and perfused retina is highly standardized and seems to be well suited for measuring immediate ERG changes and if necessary to differentiate between short-term effect and prolonged toxicity by monitoring amplitudes during the washout [18]. To leave a safety margin, we decided to test quite long staining times (60 and 120 s). The usual staining time used intraoperatively is generally shorter than 1 min.

In the model of isolated and perfused bovine retina, we could not detect significant reductions of the ERG-amplitudes after exposure to the lutein-based dye Retidyne™ for exposure times of 60 and 120 s respectively after the 75 min washout. After an exposure time of 120 s, we observed a transient drop of amplitudes which reached statistical significance only for a-waves. This might be explained by a blockage of the flash that is entering the perfusion chamber from above and has to pass through the stained nutrient solution. Our results are consistent with a study of Furlani et al. [29]. They tested lutein and zeaxanthin (L/Z) mixed with various concentrations of brilliant blue in a rabbit model and found no significant differences in ERG as well as light and electron microscopy evaluation. ERGs were performed at 24 h and 7 days after injection. Compared with our study, rabbit

retinas were in contact with different mixtures of the dyes over a longer period of time. However, the dyes were diluted by injecting them into the vitreous and the distribution in the vitreous cavity was not homogeneous. In a different study, ERG recordings were taken at baseline (before injection) and 7 days after intravitreal injection of a mixture of lutein/zeaxanthin with or without brilliant blue [30]. The authors observed major variability in the ERG results, but no variation above or below 50% between baseline and endpoint. They also performed a cytotoxicity assay and histopathologic examinations which showed no affection of the integrity of the neurosensory retina, the RPE, or the choriocapillaris-choroid complex. They concluded that dye solutions based on L/Z have a safe profile.

To provide a green staining color and therefore increase contrast against the choroid, Retidyne™ is composed of 2% lutein (orange color) and 0.05% brilliant blue (blue color). Brilliant blue is commonly applied in a higher concentration of 0.25% if it is used as a single dye for staining the ILM. It is available in the European Union market as ILM Blue (manufactured by DORC Zuidland, the Netherlands) and has shown a good safety profile even under endoillumination [31–33]. Brilliant blue in a low concentration of 0.05% can therefore be regarded as safe for intravitreal use.

The recent experiences concerning AV17 as well as the lessons learned after the introduction of ICG illustrate the persisting safety lack in the area of vital dyes and the ongoing need for multimodal preclinical testing.

In our study, we tested a lutein-based dye which is CE labeled and already in clinical use. We chose exposure times of 60 and 120 s to reflect the intraoperative situation and leave a safety margin to commonly used rather shorter exposure times. Longer exposure times were not tested in our model because there is a risk of artificially decreasing ERG amplitudes due to a longer cessation of the perfusion with nutrient solution. We can therefore not provide information about other exposure times than the tested ones.

We think that our results in a highly standardized ERG model can further substantiate the good biocompatibility of lutein-based dyes in the context of vitreoretinal procedures like ILM peeling and therefore contribute to a safer clinical application. Although, it needs to be stated that we used an ex vivo setup and testing on in vivo models could yield additional information.

Lutein as a natural component of the central retina might even have neuroprotective properties against phototoxicity to the retina caused by intraoperatively used endoillumination. Especially short wavelength irradiation such as blue light is hazardous as it can lead to damage at the photoreceptor level [34]. Lutein absorbs blue light at wavelengths around 450 nm with a peak of absorption at 446 nm. Furthermore, it also acts as an antioxidant [35]. This is in contrast to ICG which is more toxic under endoillumination by a photosensitized mechanism

[36, 37]. However, a neuroprotective value of lutein against light-induced toxicity cannot be proven in our experimental model as we have to keep the ex vivo retinal tissue in a dark-adapted state. Therefore, a possible protective value of lutein regarding toxicity caused by endoillumination would be an interesting topic for future studies.

Compliance with ethical standards

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Conflict of interest The authors declare that they have no conflict of interest.

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References

1. Luke J, Ludeke I, Acksteiner A, Nassar K, Hoerauf H, Grisanti S, Luke M (2013) Morphological and functional outcome after brilliant blue G-assisted macular hole surgery. Ophthalmologica Journal international d'ophtalmologie International journal of ophthalmology Zeitschrift fur Augenheilkunde 230(2):81–86. https://doi.org/10.1159/000351658

2. Vote BJ, Russell MK, Joondeph BC (2004) Trypan blue-assisted vitrectomy. Retina (Philadelphia, Pa) 24(5):736–738

3. Wolf S, Reichel MB, Wiedemann P, Schnurrbusch UE (2003) Clinical findings in macular hole surgery with indocyanine green-assisted peeling of the internal limiting membrane. Graefes Arch Clin Exp Ophthalmol 241(7):589–592. https://doi.org/10.1007/s00417-003-0673-1

4. Stalmans P, Van Aken EH, Veckeneer M, Feron EJ, Stalmans I (2002) Toxic effect of indocyanine green on retinal pigment epithelium related to osmotic effects of the solvent. Am J Ophthalmol 134(2):282–285 doi: S000293940201468X [pii]

5. Lee JE, Yoon TJ, Oum BS, Lee JS, Choi HY (2003) Toxicity of indocyanine green injected into the subretinal space: subretinal toxicity of indocyanine green. Retina (Philadelphia, Pa) 23(5):675–681

6. Cheng SN, Yang TC, Ho JD, Hwang JF, Cheng CK (2005) Ocular toxicity of intravitreal indocyanine green. J Ocul Pharmacol Ther 21(1):85–93. https://doi.org/10.1089/jop.2005.21.85

7. Enaida H, Sakamoto T, Hisatomi T, Goto Y, Ishibashi T (2002) Morphological and functional damage of the retina caused by intravitreous indocyanine green in rat eyes. Graefes Arch Clin Exp Ophthalmol 240(3):209–213. https://doi.org/10.1007/s00417-002-0433-7

8. von Jagow B, Hoing A, Gandorfer A, Rudolph G, Kohnen T, Kampik A, Haritoglou C (2009) Functional outcome of indocyanine green-assisted macular surgery: 7-year follow-up. Retina (Philadelphia, Pa) 29(9):1249–1256. https://doi.org/10.1097/IAE.0b013e3181a91dd3

9. Narayanan R, Kenney MC, Kamjoo S, Trinh TH, Seigel GM, Resende GP, Kuppermann BD (2005) Toxicity of indocyanine green (ICG) in combination with light on retinal pigment epithelial cells and neurosensory retinal cells. Curr Eye Res 30(6):471–478. https://doi.org/10.1080/02713680590959312

10. Kernt M, Hirneiss C, Wolf A, Liegl R, Rueping J, Neubauer A, Alge C, Ulbig M, Gandorfer A, Kampik A, Haritoglou C (2012) Indocyanine green increases light-induced oxidative stress,

senescence, and matrix metalloproteinases 1 and 3 in human RPE cells. Acta Ophthalmol 90(6):571–579. https://doi.org/10.1111/j.1755-3768.2010.01961.x

11. Bone RA, Landrum JT, Tarsis SL (1985) Preliminary identification of the human macular pigment. Vis Res 25(11):1531–1535

12. Nolan JM, Stack J, Mellerio J, Godhinio M, O'Donovan O, Neelam K, Beatty S (2006) Monthly consistency of macular pigment optical density and serum concentrations of lutein and zeaxanthin. Curr Eye Res 31(2):199–213. https://doi.org/10.1080/02713680500514677

13. Liu R, Wang T, Zhang B, Qin L, Wu C, Li Q, Ma L (2015) Lutein and zeaxanthin supplementation and association with visual function in age-related macular degeneration. Invest Ophthalmol Vis Sci 56(1):252–258. https://doi.org/10.1167/iovs.14-15553

14. Yu CC, Nandrot EF, Dun Y, Finnemann SC (2012) Dietary antioxidants prevent age-related retinal pigment epithelium actin damage and blindness in mice lacking alphavbeta5 integrin. Free Radic Biol Med 52(3):660–670. https://doi.org/10.1016/j.freeradbiomed.2011.11.021

15. Kim SR, Nakanishi K, Itagaki Y, Sparrow JR (2006) Photooxidation of A2-PE, a photoreceptor outer segment fluorophore, and protection by lutein and zeaxanthin. Exp Eye Res 82(5):828–839. https://doi.org/10.1016/j.exer.2005.10.004

16. Kijlstra A, Tian Y, Kelly ER, Berendschot TT (2012) Lutein: more than just a filter for blue light. Prog Retin Eye Res 31(4):303–315. https://doi.org/10.1016/j.preteyeres.2012.03.002

17. Badaro E, Furlani B, Prazeres J, Maia M, Lima AA, Souza-Martins D, Muccioli C, Lucatto LF, Belfort R Jr (2014) Soluble lutein in combination with brilliant blue as a new dye for chromovitrectomy. Graefes Arch Clin Exp Ophthalmol 252(7):1071–1078. https://doi.org/10.1007/s00417-013-2539-5

18. Luke M, Weiergraber M, Brand C, Siapich SA, Banat M, Hescheler J, Luke C, Schneider T (2005) The isolated perfused bovine retina–a sensitive tool for pharmacological research on retinal function. Brain Res Brain Res Protoc 16(1–3):27–36. https://doi.org/10.1016/j.brainresprot.2005.09.001

19. Sickel W (1966) The isolated retina maintained in a circulating medium. Combined optical and electrical investigations of metabolic aspects of the generation of the electroretinogram. In: Clinical electroretinography. Pergamon Press, Oxford, pp 115–124

20. Kadonosono K, Itoh N, Uchio E, Nakamura S, Ohno S (2000) Staining of internal limiting membrane in macular hole surgery. Arch Ophthalmol 118(8):1116–1118

21. Wollensak G (2008) Biomechanical changes of the internal limiting membrane after indocyanine green staining. Dev Ophthalmol 42:82–90. https://doi.org/10.1159/000138975

22. Rodrigues EB, Meyer CH (2008) Meta-analysis of chromovitrectomy with indocyanine green in macular hole surgery. Ophthalmologica Journal international d'ophtalmologie International journal of ophthalmology Zeitschrift fur Augenheilkunde 222(2):123–129. https://doi.org/10.1159/000112630

23. Wu Y, Zhu W, Xu D, Li YH, Ba J, Zhang XL, Wang F, Yu J (2012) Indocyanine green-assisted internal limiting membrane peeling in macular hole surgery: a meta-analysis. PLoS One 7(11):e48405. https://doi.org/10.1371/journal.pone.0048405

24. Thaler S, Schuttauf F, Haritoglou C (2009) Biocompatibility of dyes for vitreoretinal surgery. Der Ophthalmologe : Zeitschrift der Deutschen Ophthalmologischen Gesellschaft 106(1):11–15. https://doi.org/10.1007/s00347-008-1854-4

25. Tura A, Alt A, Haritoglou C, Meyer CH, Schneider T, Grisanti S, Luke J, Luke M, International Chromovitrectomy C (2014) Testing the effects of the dye acid violet-17 on retinal function for an intraocular application in vitreo-retinal surgery. Graefes Arch Clin Exp Ophthalmol 252(12):1927–1937. https://doi.org/10.1007/s00417-014-2761-9

26. Gerding H (2017) Intraocular use of acid violet 17 at a concentration of 1.5 mg/ml is not safe. Graefes Arch Clin Exp Ophthalmol 255(3):627–628. https://doi.org/10.1007/s00417-016-3574-9

27. Gerding H (2016) Acid violet 17: a new dye for chromovitrectomy? Klin Monatsbl Augenheilkd 233(4):460–464. https://doi.org/10.1055/s-0041-111823

28. Hurst J, Schnichels S, Spitzer MS, Bartz-Schmidt KU, Farecki ML, Szurman P, Januschowski K (2017) Negative effects of acid violet-17 and MBB dual in vitro on different ocular cell lines. Curr Eye Res 42:1–6. https://doi.org/10.1080/02713683.2017.1285942

29. Furlani BA, Barroso L, Sousa-Martins D, Maia M, Moraes-Filho MN, Badaro E, Portella R, Lima-Filho AA, Rodrigues EB, Belfort R Jr (2014) Lutein and zeaxanthin toxicity with and without brilliant blue in rabbits. J Ocul Pharmacol Ther 30(7):559–566. https://doi.org/10.1089/jop.2013.0171

30. Casaroli-Marano RP, Sousa-Martins D, Martinez-Conesa EM, Badaro E, Nunes RP, Lima-Filho AA, Rodrigues EB, Belfort R Jr, Maia M (2015) Dye solutions based on lutein and zeaxanthin: in vitro and in vivo analysis of ocular toxicity profiles. Curr Eye Res 40(7):707–718. https://doi.org/10.3109/02713683.2014.952831

31. Henrich PB, Haritoglou C, Meyer P, Ferreira PR, Schotzau A, Katamay R, Josifova T, Schneider U, Flammer J, Priglinger S (2010) Anatomical and functional outcome in Brilliant Blue G assisted chromovitrectomy. Acta Ophthalmol 88(5):588–593. https://doi.org/10.1111/j.1755-3768.2008.01477.x

32. Hoing A, Remy M, Dirisamer M, Priglinger S, Schonfeld CL, Kampik A, Haritoglou C (2011) An in-vivo evaluation of Brilliant Blue G in macular surgery. Klin Monatsbl Augenheilkd 228(8):724–728. https://doi.org/10.1055/s-0031-1273351 [doi]

33. Mansoor S, Sharma A, Caceres-Del-Carpio J, Zacharias LC, Patil AJ, Gupta N, Limb GA, Kenney MC, Kuppermann BD (2015) Effects of light on retinal pigment epithelial cells, neurosensory retinal cells and Muller cells treated with Brilliant Blue G. Clin Exp Ophthalmol 43(9):820–829. https://doi.org/10.1111/ceo.12568

34. van den Biesen PR, Berenschot T, Verdaasdonk RM, van Weelden H, van Norren D (2000) Endoillumination during vitrectomy and phototoxicity thresholds. Br J Ophthalmol 84(12):1372–1375

35. Sundelin SP, Nilsson SE (2001) Lipofuscin-formation in retinal pigment epithelial cells is reduced by antioxidants. Free Radic Biol Med 31(2):217–225

36. Kwok AK, Lai TY, Yeung CK, Yeung YS, Li WW, Chiang SW (2005) The effects of indocyanine green and endoillumination on rabbit retina: an electroretinographic and histological study. Br J Ophthalmol 89(7):897–900. https://doi.org/10.1136/bjo.2004.061093

37. Glickman RD (2002) Phototoxicity to the retina: mechanisms of damage. Int J Toxicol 21(6):473–490. https://doi.org/10.1080/10915810290169909