Comparing the Mode of Action of Intraocular Lutein-Based Dyes With Synthetic Dyes

Diogo Sousa-Martins,[1,2] Luciano Caseli,[3] Mafalda C. Figueiredo,[1] Claudia Sa e Cunha,[1] Helder Mota-Filipe,[1] Acácio Souza-Lima,[2] Rubens Belfort Jr,[2] Eduardo Rodrigues,[2] and Mauricio Maia[2]

[1]Department of Social Pharmacy, College of Pharmacy, University of Lisbon, Lisbon, Portugal
[2]Department of Ophthalmology, Escola Paulista de Medicina, Federal University of São Paulo (Unifesp), São Paulo, Brazil
[3]Department of Earth and Exacts Sciences, Federal University of São Paulo (Unifesp), Diadema, São Paulo, Brazil

Correspondence: Diogo Sousa-Martins, Department of Social Pharmacy, College of Pharmacy, University of Lisbon, Avenida Professor Gama Pinto, 1649-003 Lisbon, Portugal; dsousamartins@gmail.com.

Submitted: December 2, 2014
Accepted: January 17, 2015

Citation: Sousa-Martins D, Caseli L, Figueiredo MC, et al. Comparing the mode of action of intraocular lutein-based dyes with synthetic dyes. *Invest Ophthalmol Vis Sci*. 2015;56:1993–2000. DOI:10.1167/iovs.14-16187

Purpose. To investigate and compare the mechanism by which lutein-based and synthetic intraocular dyes interact with their target membranes during ophthalmic surgeries.

Methods. Surrogate membrane models were used in order to simulate the different intraocular membranes: internal limiting membrane (ILM), vitreous, anterior capsule (AC), and epiretinal membrane (ERM). Different lutein-based dyes, such as Phacodyne, Retidyne, Retidyne Plus, and Vitreodyne were tested, as well as Trypan Blue (TB), Indocyanine Green (ICG), Brilliant Blue (BB), and Triamcinolone Acetonide (TA). The interactions between the film components occurring at the air–water interface were investigated with surface pressure–area isotherms and polarization modulation infrared reflection-absorption spectroscopy (PM-IRRAS).

Results. With the exception of TA and ICG, none of the tested dyes revealed toxicity to the analyzed membranes. The interaction of TA with the vitreous model affected deeply the biointerface structure of the model. A significant condensation of the monolayer is noted when ICG contacted with ILM by the isotherms or even a solubilization of part of the monolayer toward the aqueous subphase. Retidyne Plus may provide the fluidization of the membrane, but maintains intact the structure of proteins present in the model.

Conclusions. The present study demonstrates for the first time that lutein-based dyes interact through a physical mechanism of action with membrane models of structures present in human eye. On the other hand, the chemical interaction of synthetic dyes TA and ICG resulted in an alteration of the membrane models.

Keywords: lutein-based dyes, synthetic dyes, intraocular membranes, Langmuir films

Intraoperative application of vital dyes for the visualization of intraocular membranes and tissues has facilitated surgical techniques and outcomes in recent years.[1,2] In cataract surgery, the blue dye Trypan Blue (TB) gained widespread use because it stains the anterior capsule (AC) and enables easier intraoperative removal of this fine, semitransparent membrane.[1] In vitreoretinal surgery, green and blue vital dyes such as Indocyanine Green (ICG) and Brilliant Blue (BB) facilitate identification and removal of the internal limiting membrane (ILM) as a result of their different affinities to intraocular collagen and cellular elements.[2,3] Triamcinolone Acetonide (TA) has been used most often to facilitate identification of the posterior hyaloid and vitreous.[4] However, all these dyes are synthetic and may present some toxicity profile that may limit their use in ocular surgery, especially vitreoretinal surgery.[2,5–10]

Lutein and zeaxanthin (L/Z) are lipophilic pigments belonging to the group of carotenoids traditionally found in fruit and vegetables. In addition, L/Z have been identified as the major components of macular pigment.[11,12] Published peer-reviewed studies have reported association of L/Z with prevention of age-related maculopathies due to an antioxidant and blue light filtering mechanisms.[13–18] In addition, Lutein has been recently associated with potential neuroprotective effects in the retina.[19–21]

In our previous publications, we have described the use of novel intraocular dyes in which Lutein is the primary component in cataract and vitreoretinal surgeries.[22–28] These L/Z-based dyes have shown efficacy in staining target membranes such as AC, ILM, and the vitreous, as well as an excellent safety profile.[22–28]

The nature of the interaction of L/Z-based dyes with their target membranes is herein investigated using Langmuir monolayer models simulating the ILM, vitreous, AC, and epiretinal membrane (ERM). This methodology is justified since monomolecular films at the air–water interface are widely recognized to be systems able to mimic biological membranes and other biological surfaces.[29–33] We have previously created a model of human ILM using biointerfaces to analyze the mechanisms by which vital dyes stain the ILM that allowed evaluating the intermolecular interactions of a L/Z-based dye in such experimental mode.[23] The purpose of this study was to evaluate the intermolecular interactions of L/Z-based dyes and the synthetic dyes, TB, BB, ICG, and TA, with their respective target membranes during ocular surgery. Surrogate membrane models of dipalmitoyphosphatidylcholine (DPPC) Langmuir monolayers were used, pure or mixed with different components, in order to simulate the different intraocular membranes.

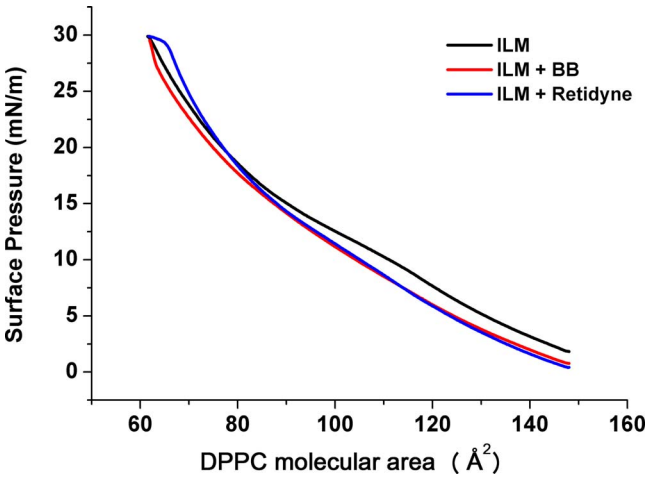

FIGURE 1. Surface pressure–area isotherms mimicking the ILM of human eyes in absence or presence of the dyes BB and Retidyne.

METHODS

All the materials used in this experimental study were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA) unless otherwise stated.

For all monolayers, DPPC was applied as lipid model. Surface pressure–area (π-A) isotherms were obtained through a mini Langmuir trough (KSV Instruments Ltd., Espoo, Finland) equipped with a surface pressure sensor based on the Wilhelmy method. Movable barriers that sweep the air–water interface with a rate of 5 Å^2 molecule^{-1} minute^{-1} were employed to compress the monolayers. Initially, the Langmuir trough was filled with a buffer solution (phosphate 1.0 mM, NaCl 100 mM, pH = 7.2), and then a DPPC solution at a 0.5 mg/mL concentration was carefully spread on the air–water interface drop-by-drop. After 20 minutes elapsed for chloroform evaporation, compression of the monolayer was then carried out, and the surface pressure (π), defined as $\gamma_0 - \gamma$, being γ_0, and γ the surface tension of the aqueous subphase without and with the covering of the monolayer, respectively, was followed as long as the average molecular area (A) of DPPC decreased. The π-A curves were obtained at least three times to test the reproducibility of the experiments.

Other biological components present in ILM, vitreous, AC, and ERM were also evaluated in this study, and different combinations of DPPC with a second component were carried out in order to better investigate the role of each one. For that, aliquots of 5 µL of a 0.5 mg/mL solution of each component were cospread with DPPC. The following components were tested: (1) collagen type IV from human placenta, dissolved in 0.25% acetic acid, phosphate 0.1 mM, NaCl 100 mM, pH = 7.2; (2) laminin from Engelbreth-Holm-Swarm murine sarcoma basement membrane, dissolved in phosphate 1.0 mM, NaCl 100 mM, pH = 7.2; and (3) proteoglycan from bovine nasal septum, dissolved in phosphate 1.0 mM, NaCl 100 mM, pH = 7.2. For the surrogate membranes, the main components from each system were used in order to mimic the desired biosurface: (1) ILM: DPPC, collagen, laminin, and proteoglycan; (2) AC: collagen and proteoglycan; (3) ERM: DPPC; and (4) vitreous: collagen. For the components that did not present surface activity, instead of spreading the materials on the air–water interface they were casted on silicon wafers. After that, all the related components were mixed together and cospread with DPPC in order to observe the overall effect of the dye on the model.

The L/Z-based dyes (all from Kemin Pharma, Barcarena, Portugal) Phacodyne (L/Z 1% + TB 0.04%), Retidyne (L/Z 2% + BB 0.05%), Retidyne Plus (L/Z 1.8% + BB 0.05%), and Vitreodyne (L/Z 2%) were inserted in the films, with all possible combinations, in aliquots of 5 µL. The synthetic dyes, BB 0.05% (Sigma-Aldrich Corp.), TB (Merck KGaA, Darmstadt, Germany), ICG (AlfaIntes, Casori, Italy), and TA (Farmabios, Gropello Cairopoli, Italy) were mixed where applicable with ophthalmic suitable vehicles, sterilized, packed into vials, and tested in these models. After dye incorporation in the monolayer, the measurements were performed only after 30 minutes in order to allow stabilization of the film in terms of lateral diffusion and homogenization.

Additionally, polarization modulation infrared reflection-absorption spectroscopy (PM-IRRAS) measurements were carried out with a KSV PMI 550TM, (KSV Instruments Ltd.). The Langmuir trough was set up so that the light beam was able to reach the monolayer at a fixed incidence angle of 80°. At this angle, the light beam intensity was the maximum and the noise level was the lowest. The incoming light was continuously modulated between s- and p-polarization at a high frequency, which allowed for the simultaneous measurement of the spectrum for the two polarizations. The difference

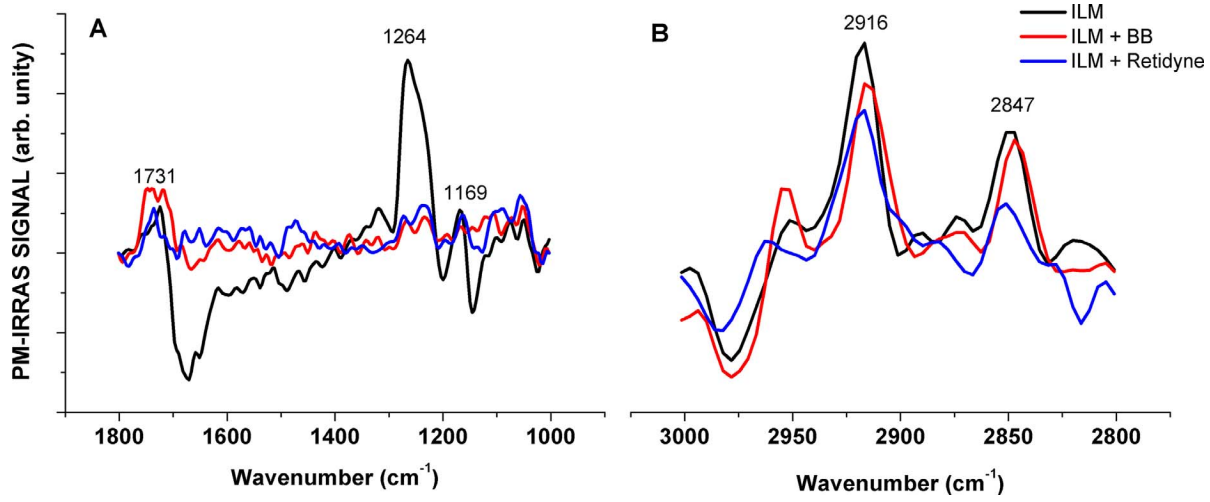

FIGURE 2. Polarization modulation infrared reflection-absorption spectroscopy spectra for ILM in absence or presence of the dyes BB and Retidyne. (**A**) Hydrophilic region (region between 1000 and 1800 cm−1); (**B**) hydrophobic region (region between 2800 and 3000 cm^{-1}).

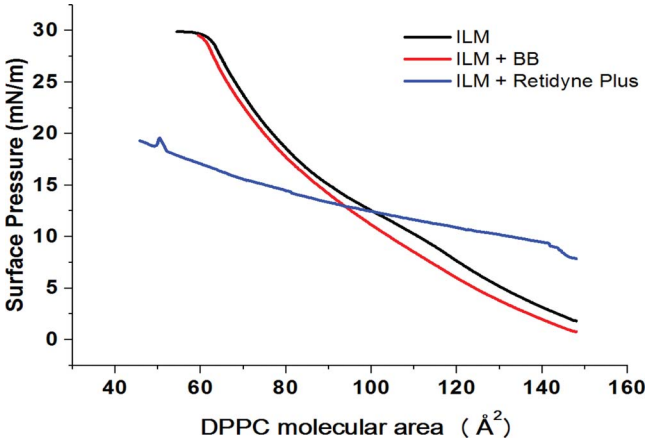

FIGURE 3. Surface pressure–area isotherms for ILM in absence or presence of the dyes BB and Retidyne Plus.

between the spectrum provided surface-specific information, and the sum provided the reference spectrum; using the simultaneous measurements, the effect of water vapor was largely reduced.

All the experiments were carried out at a controlled room temperature (25.0 ± 0.1°C). All the other reagents were the highest purity available. Water employed was previously purified with Milli-Q system, with resistivity of 18.2 MΩ cm and pH of 5.4.

RESULTS

Interactions of Ophthalmological Dyes With Surrogate Internal Limiting Membrane

In order to investigate the mechanism by which lutein-based and synthetic ocular dyes interact with ILM during ophthalmic surgeries, we used a surrogate membrane model for ILM. To that end, the interactions between the film components and the ophthalmic dyes were investigated with surface pressure–area isotherms and PM-IRRAS.

We started by examining the mode of action of Retidyne and BB. Figure 1 shows that both dyes shift the isotherms to lower molecular area comparing with the isotherm for ILM without dyes. This shift is related to the condensation of the monolayer probably caused by interaction between the dyes and the polar groups of the monolayer. Comparing the effect of the two dyes studied, there is no significant difference, and for both there is a first indication of an intermolecular interaction with the target membrane. During the present study, we have also observed that lutein was the primary component in staining the ILM membrane.

The PM-IRRAS spectra for ILM indicate that the hydrophilic region of this membrane is significantly affected upon the presence of both dyes (Fig. 2A). This is probably due to a high affinity of the dyes for hydrophilic region of the membrane. The carbonyl stretch band, centered in 1731 cm^{-1}, is split in two bands in the presence of Retidyne and is shifted to higher wavenumbers with BB. The negative band around 1680 cm^{-1} is attributed to the difference of reflectivity of the water surface with and without the presence of the monolayer, and may reflect the degree of hydration and orientation of water molecules in contact with the monolayer. This band is absent for both dyes, which indicates that they may affect the water molecules that blind the polar heads of the monolayer. The bands centered in 1264 and 1169 cm^{-1} may reflect the C-O-C vibration for the polysaccharides present in the model for ILM, and must overlap the phosphate band for DPPC usually present in this region, but with a lower intensity. With the presence of both dyes, these bands are no longer well defined maybe because of the high affinity of the dye for the glycidic part of the ILM, which reflects the hydrophilicity of the dyes. For the hydrophobic part, where some vibrations for the alkyl chains of DPPC are shown (Fig. 2B), some effect is also observed, as shifts of the maxima, and appearance of a shoulder in approximately 2950 cm^{-1}, reflecting CH_3 vibrations. Comparing to the effect in the hydrophilic parts (Fig. 2A) the situation observed when the dyes are introduced is not so evident. These changes observed may reflect therefore the disorganization of the alkyl chains caused by the interaction with the polar heads of the phospholipid.

Next, we compare the interaction of Retidyne Plus and BB with the surrogate ILM. As depicted in Figure 3, isotherms for ILM models in the presence of Retidyne Plus show a remarkable change characterized by a film highly compressible. On the other hand, the presence of the synthetic dye BB does not trigger any significant change in the model studied when compared with the isotherm without dyes.

The Retidyne Plus effect in the hydrophilic region (Fig. 4A) is similar to that observed for BB and Retidyne (Figs. 1, 2). However, for the hydrophobic region the effect is relatively more pronounced when compared with the effect from the other dyes (Fig. 4B). The band becomes larger corroborating

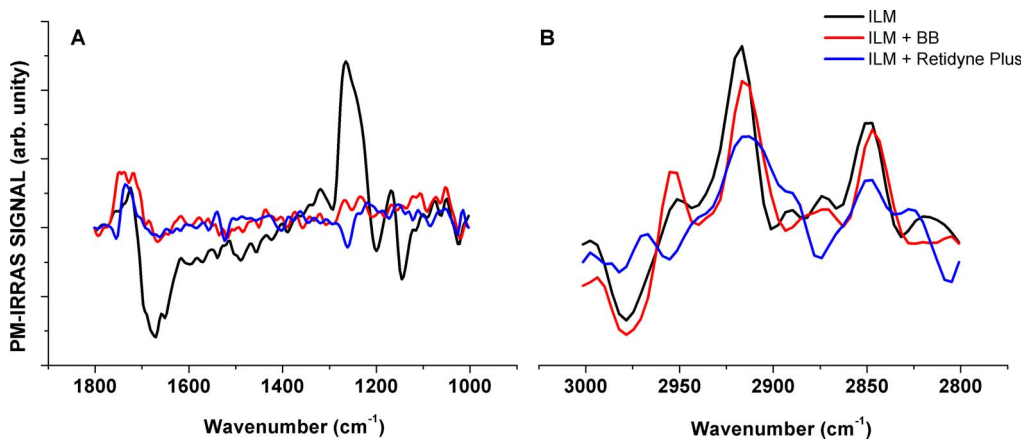

FIGURE 4. Polarization modulation infrared reflection-absorption spectroscopy spectra for ILM in absence or presence of the dyes BB and Retidyne Plus. (**A**) Hydrophilic region (region between 1000 and 1800 cm^{-1}); (**B**) hydrophobic region (region between 2800 and 3000 cm^{-1}).

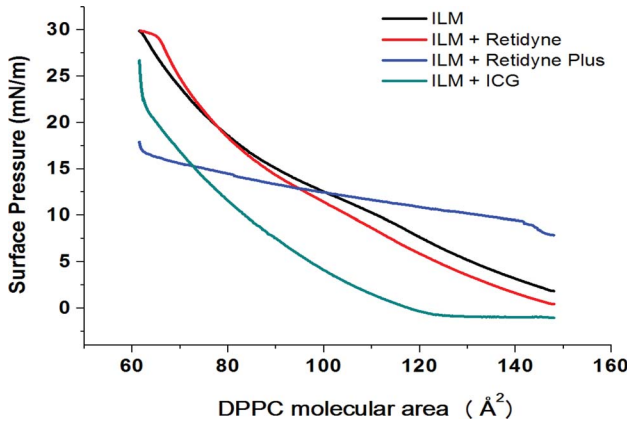

FIGURE 5. Surface pressure–area isotherms for ILM in absence or presence of the dyes Retidyne, Retidyne Plus, and the synthetic dye ICG.

the statement obtained in the π-A isotherm in which this dye disorganizes the ILM monolayer, which in turn reflect in the molecular disorganization for the hydrophobic region. This indicates a specific interaction between Retidyne Plus and this region of the membrane.

We have also compared the interaction of Retidyne, Retidyne Plus, and ICG in a surrogate ILM. We found a marked effect of ICG when compared with other dyes (Fig. 5). In comparison with Retidyne, while for the latter a small shift to lower areas is observed, for ICG a significant condensation of the monolayer is noted by the isotherms or even a solubilization of part of the monolayer toward the aqueous subphase. Probably this effect may be directly related to the amphiphilic character of this dye that may facilitate the solubilization of ILM into the aqueous phase.

The polarization modulation analyze shows that the band for water increased with ICG and the band for symmetric C-H stretches for CH_2 disappeared, with a larger band in 2867 cm^{-1}, related to CH_3 being shown (Fig. 6). These data demonstrate that water may be replaced in the monolayer due to solubilization of part of the monolayer, which reflects the positive band in approximately 1680 cm^{-1}. This may provide a remarkable molecular disorganization in the monolayer, which reflects in the bands presented in the C-H stretching mode region. All dyes revealed an affinity to ILM, interacting with both hydrophobic and hydrophilic parts of the membrane, but with ICG we observe a higher effect in terms of disintegration of the membrane.

Comparison of Triamcinolone, Vitreodyne, and Retidyne Plus in a Vitreous Model

The first attempt for making a vitreous model did not present surface activity and it was not possible to build a model at the air–water interface. Therefore, the model was created on silicon wafers, with collagen being casted on them. We analyzed the behavior of this model when in contact with TA, Vitreodyne, and Retidyne Plus dyes. None of the studied dyes presented significant effect on the amide I band (~1653 cm^{-1}; Fig. 7A). However, a discrete alteration of the order of methyl bands was observed as seen in Figure 7B. This could be related to the effect of the methyl groups of the dyes. However, in Figure 7A, a band of approximately 1620 cm^{-1}, usually related to beta-sheets appears when TA is inserted, points to a more significant effect of this dye, which may disrupt the protein structure of this surface. The other dyes do not present any significant effect, which indicates that these dyes do not alter the chemical structures of the molecules that form the model for the membrane.

Interaction of Ophthalmic Dyes With the Anterior Capsule Model

Similarly to what happened with vitreous model, the AC model did not present surface activity and it was not possible to build a model at the air–water interface. For that reason, the model was created on silicon wafers, with collagen and proteoglycan being casted on them.

The analyzed dyes (TB and Phacodyne) did not interact with the protein and glicid structure of the models in such a way that they do not denature the structure of these biomolecules (Fig. 8A). Figure 8B shows that both can interact with the model because the spectra for the methyl stretch region is changed upon the introduction of both dyes.

Comparison of Phacodyne With Trypan Blue in a Surrogate Epiretinal Membrane

Isotherms in Figure 9 show that both TB and Phacodyne penetrate in the ERM because they shift the isotherms to higher areas. The effect from Phacodyne is more pronounced, indicating a higher affinity of Phacodyne for this membrane.

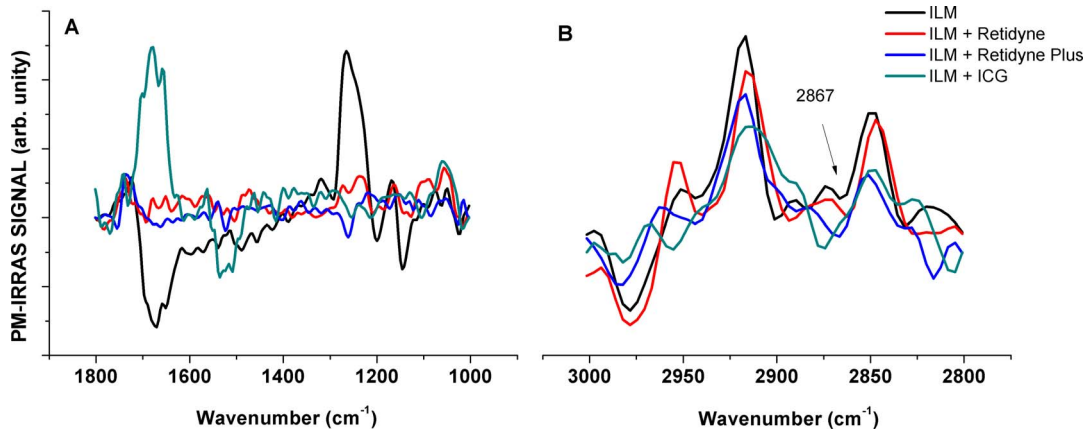

FIGURE 6. Polarization modulation infrared reflection-absorption spectroscopy spectra for ILM in absence or presence of the natural dyes Retidyne, Retidyne Plus, and synthetic ICG. (**A**) Hydrophilic region (region between 1000 and 1800 cm^{-1}); (**B**) hydrophobic region (region between 2800 and 3000 cm^{-1}).

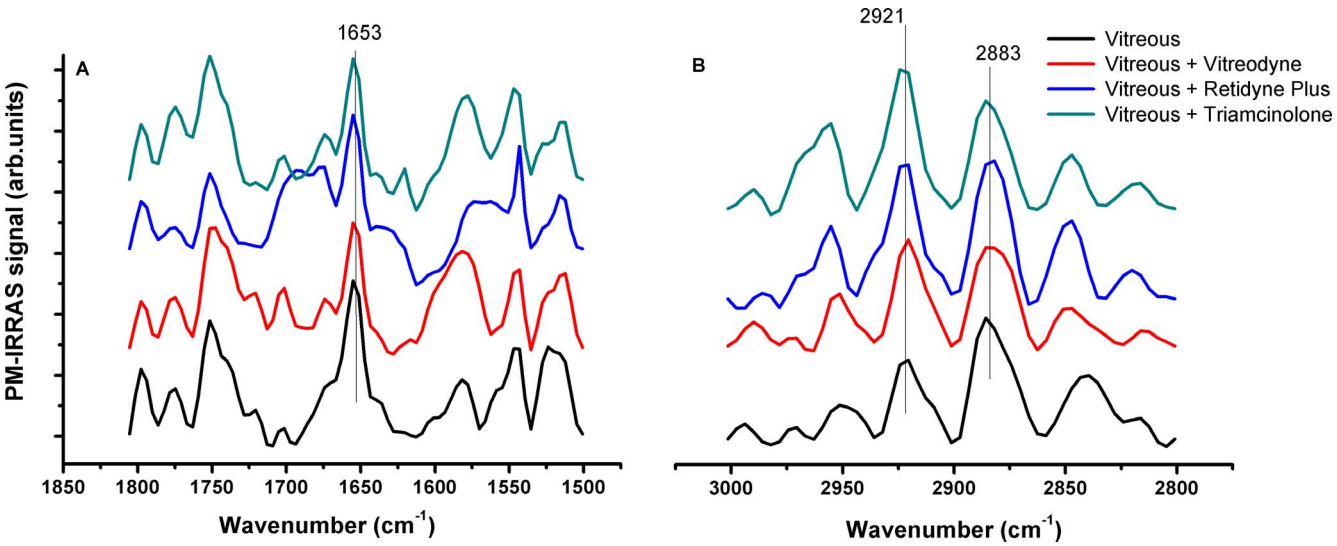

FIGURE 7. Polarization modulation infrared reflection-absorption spectroscopy spectra mimicking the vitreous of the human eyes in absence or presence of the Vitreodyne, Retidyne Plus, and Triamcinolone dyes. (**A**) Hydrophilic region (region between 1000 and 1800 cm−1); (**B**) hydrophobic region (region between 2800 and 3000 cm^{-1}).

Trypan Blue and Phacodyne change significantly the PM-IRRAS spectra for ERM model (Fig. 10). The carbonyl band for DPPC is shifted to higher wavenumbers, the negative band for surface water disappears, and the bands at approximately 1250 and 1180 cm^{-1} are more evident (Fig. 10A). Also for the CH stretches, the band centered in 2945 cm^{-1} become thinner, and the relative intensities between asymmetric and symmetric band are changed (Fig. 10B). This shows a clear interaction between the dyes and this membrane without evidence of dye–membrane covalent bonds. Furthermore, we have also observed that of all the components of the product tested, lutein was the primary component to stain the ERM.

DISCUSSION

In this work we used surrogate models simulating ILM, vitreous, AC, and ERM in order to compare the interaction between natural and synthetic intraocular dyes with the biointerfaces for which these compounds take action during ophthalmic surgeries.

The interaction of Retidyne and BB with ILM revealed no significant alterations in the isotherms of this membrane in terms of surface elasticity. Specifically for Retidyne, these results suggest a nonchemical bonding to the membrane and are in agreement with previous preclinical and clinical tests that showed that this natural dye is safe to be used intraocularly.[22,24,26,28] However, the results for BB do not correlate completely with reports that showed dose- and time-dependent toxicity of this dye in retinal cell lines, revealing that it is prudent to use the lowest possible concentration during the surgery.[34,35] When analyzing the behavior of Retidyne Plus while in contact with the same model, we observed a change in the isotherm of ILM model. These results are in agreement with those ones previously reported [23] for ILM, but with a lower amount of Retydine Plus. In that study, an isotherm with a high compressibility was also shown, as an indicative of successful adherence of this dye to the membrane. Other studies showed that this ophthalmic dye showed no toxicity when tested in in vitro and in vivo conditions. The citotoxicity of this dye was evaluated using cell models of retinal pigment epithelial cells (ARPE-19) and human

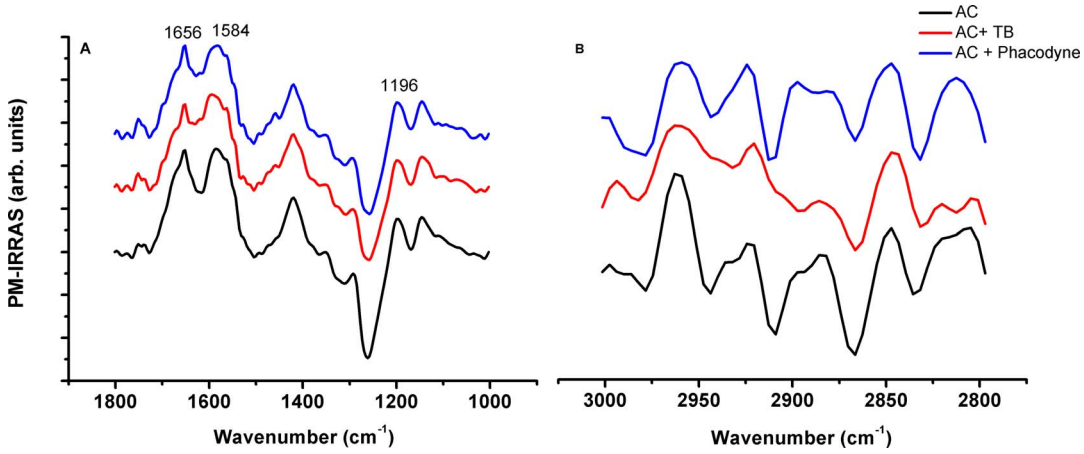

FIGURE 8. Polarization modulation infrared reflection-absorption spectroscopy spectra mimicking the anterior capsule of the human eyes in absence or presence of the dyes TB and Phacodyne. (**A**) Hydrophilic region (region between 1000 and 1800 cm−1); (**B**) hydrophobic region (region between 2800 and 3000 cm^{-1}).

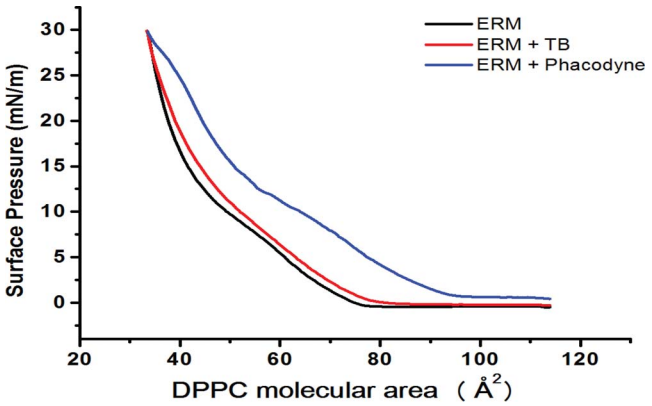

FIGURE 9. Surface pressure–area isotherms, mimicking the ERM of human eyes, in absence or presence of the TB and Phacodyne dyes.

corneal epithelial cells (HCE), as well as, in rabbits, revealing no dye-related cytotoxicity.[24,28] Moreover, the safety of this dye was also tested in patients that underwent pars plana vitrectomy without clinical side effects, showing, therefore, that this lutein-based dye has a safe profile to intraocular use.[25] This fact indicates that the fluidization of the membrane observed by this dye as indicated by the isotherms and vibrational spectra is not related to a possible toxicity. The disorganization of the membrane may be an important factor for the stain of the dye. Also, for the region of amide, we do not see any significant alteration of the bands in relation to the surface representing ILM. As proteins represent a major factor for the integrity of biointerfaces, these results show that Retidyne Plus does not affect significantly the structure of these biomacromolecules. The synthetic dye ICG caused a significant condensation as well as a molecular disorganization of the ILM model monolayer. Indeed, this data can be correlated with other reports that showed the adverse effects of ICG at the retinal surface.[36–38] It is important to emphasize that this effect may be different from that for Retidyne Plus. For the latter, the fluidization of the membrane is related to the way by which the dye is incorporated to the film, altering the ability of the monolayer to be well packed molecularly. The presence of the dye may affect mainly the hydrophobic regions of the films, which may decrease the rigidity of the monolayer, but with low consequences for the structure of the film. For ICG, however, a high shift to lower areas is observed even for higher molecular areas, which may be an indicative of the solubilization of the film, leading to the loss of molecules from the air–water interface toward the aqueous subphase.

The interaction of Retidyne Plus, Vitreodyne, and TA with the vitreous model showed that when TA is inserted, the protein structure of this surface may be disrupted. This deleterious effect is in accordance with several studies that report the side effects of the intraocular use of this synthetic water-insoluble corticosteroid. Triamcinolone Acetonide is known for many different adverse effects when injected in the eye including rise of IOP, acute endophthalmitis (both infectious and noninfectious), and cataract progress.[39–45] No significant alteration of the vitreous model structure was observed when Vitreodyne and Retidyne Plus contact with the membrane. These results are consistent with the security of use these lutein-based dyes during vitreoretinal surgeries that has been already shown in preclinical and clinical studies.[25,28]

The study of Phacodyne and TB interaction with anterior capsule and ERM revealed that both dyes interact with this model without altering the molecular structure of the analyzed model. Previous reports had already shown the safety and efficacy of Phacodyne during cataract surgery.[27] Although these results indicate a similar behavior for these two dyes, other studies point to retinal toxicity of TB. Several studies have described retinal damage after exposure to TB in a bovine model, as well as, in vitro rodent neurosensory cells.[46–48] These differences may be related with the concentrations used in each study.

A limitation of this study is that these membrane models are an in vitro model that mimic the intraocular membranes present in the human eye, thus these results are only referring to the physical mechanisms of action by which these dyes interact with these membranes. However, from the results observed it is possible to speculate that our results can be correlated with the behavior that these natural and synthetic dyes have in vivo.

In conclusion, this study demonstrates that lutein-based dyes can interact with different membrane models of structures present in the human eye (ILM, vitreous, AC, and ERM). The results have also described that these dyes interact at the intermolecular level with the models affecting both hydrophilic and hydrophobic regions of the components. With the exception of TA and ICG, none of the tested dyes revealed adverse effects to the analyzed membranes corroborating the idea that is safe to use lutein-based dyes intraocularly. These experiments confirmed that the use of surface chemistry was useful to understand in detail the molecular interaction among the intraocular dyes, and the models for the membranes where these compounds act.

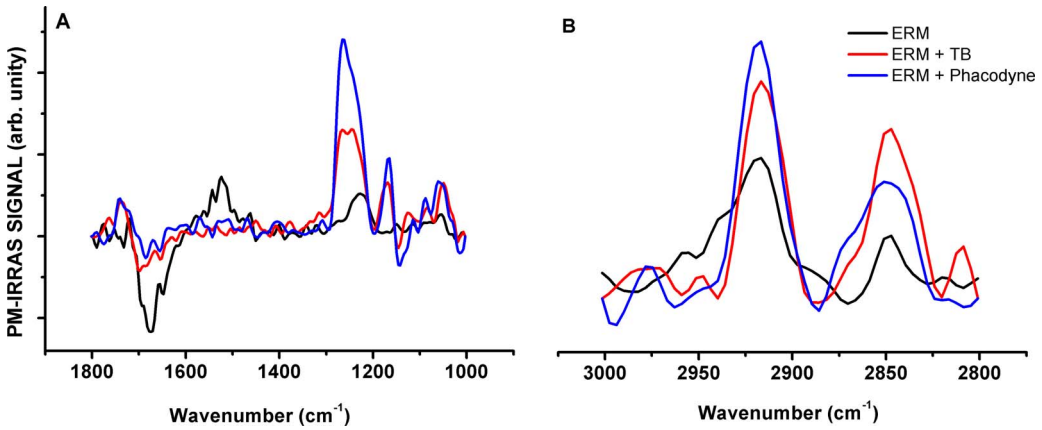

FIGURE 10. Polarization modulation infrared reflection-absorption spectroscopy spectra for ERM in absence or presence of the dyes TB and Phacodyne. (**A**) Hydrophilic region (region between 1000 and 1800 cm^{-1}); (**B**) hydrophobic region (region between 2800 and 3000 cm^{-1}).

Acknowledgments

Disclosure: **D. Sousa-Martins**, P; **L. Caseli**, None; **M.C. Figueiredo**, None; **C. Sa e Cunha**, None; **H. Mota-Filipe**, None; **A. Souza-Lima**, P; **R. Belfort Jr**, P; **E. Rodrigues**, None; **M. Maia**, P

References

1. Jacobs DS, Cox TA, Wagoner MD, et al. Capsule staining as an adjunct to cataract surgery: a report from the American Academy of Ophthalmology. *Ophthalmology*. 2006;113:707–713.
2. Rodrigues EB, Maia M, Meyer CH, Penha FM, Dib E, Farah ME. Vital dyes for chromovitrectomy. *Curr Opin Ophthalmol*. 2007;18:179–187.
3. Rodrigues EB, Meyer CH, Mennel S, Farah ME. Mechanisms of intravitreal toxicity of indocyanine green dye: implications for chromovitrectomy. *Retina*. 2007;27:958–970.
4. Peyman GA, Cheema R, Conway MD, Fang T. Triamcinolone acetonide as an aid to visualization of the vitreous and the posterior hyaloid during pars plana vitrectomy. *Retina*. 2000; 20:554–555.
5. Ando F, Yasui O, Hirose H, Ohba N. Optic nerve atrophy after vitrectomy with indocyanine green-assisted internal limiting membrane peeling in diffuse diabetic macular edema. Adverse effect of ICG-assisted ILM peeling. *Graefes Arch Clin Exp Ophthalmol*. 2004;242:995–999.
6. Farah ME, Maia M, Furlani B, et al. Current concepts of trypan blue in chromovitrectomy. *Dev Ophthalmol*. 2008;42:91–100.
7. Malerbi FK, Maia M, Farah ME, Rodrigues EB. Subretinal brilliant blue G migration during internal limiting membrane peeling. *Br J Ophthalmol*. 2009;93:1687.
8. Rodrigues EB, Costa EF, Penha FM, et al. The use of vital dyes in ocular surgery. *Surv Ophthalmol*. 2009;54:576–617.
9. Sakamoto T, Enaida H, Kubota T, et al. Incidence of acute endophthalmitis after triamcinolone-assisted pars plana vitrectomy. *Am J Ophthalmol*. 2004;138:137–138.
10. Veckeneer M, van Overdam K, Monzer J, et al. Ocular toxicity study of trypan blue injected into the vitreous cavity of rabbit eyes. *Graefes Arch Clin Exp Ophthalmol*. 2001;239:698–704.
11. Ahmed SS, Lott MN, Marcus DM. The macular xanthophylls. *Surv Ophthalmol*. 2005;50:183–193.
12. Nolan JM, Stack J, Mellerio J, et al. Monthly consistency of macular pigment optical density and serum concentrations of lutein and zeaxanthin. *Curr Eye Res*. 2006;31:199–213.
13. Beatty S, Nolan J, Kavanagh H, O'Donovan O. Macular pigment optical density and its relationship with serum and dietary levels of lutein and zeaxanthin. *Arch Biochem Biophys*. 2004; 430:70–76.
14. Bone RA, Landrum JT, Mayne ST, Gomez CM, Tibor SE, Twaroska EE. Macular pigment in donor eyes with and without AMD: a case-control study. *Invest Ophthalmol Vis Sci*. 2001;42:235–240.
15. Junghans A, Sies H, Stahl W. Macular pigments lutein and zeaxanthin as blue light filters studied in liposomes. *Arch Biochem Biophys*. 2001;391:160–164.
16. Seddon JM, Ajani UA, Sperduto RD, et al. Dietary carotenoids, vitamins A, C, and E, and advanced age-related macular degeneration. Eye Disease Case-Control Study Group. *JAMA*. 1994;272:1413–1420.
17. Sujak A, Gabrielska J, Grudzinski W, Borc R, Mazurek P, Gruszecki WI. Lutein and zeaxanthin as protectors of lipid membranes against oxidative damage: the structural aspects. *Arch Biochem Biophys*. 1999;371:301–307.
18. Sundelin SP, Nilsson SE. Lipofuscin-formation in retinal pigment epithelial cells is reduced by antioxidants. *Free Rad Biol Med*. 2001;31:217–225.
19. Ozawa Y, Sasaki M, Takahashi N, Kamoshita M, Miyake S, Tsubota K. Neuroprotective effects of lutein in the retina. *Curr Pharm Des*. 2012;18:51–56.
20. Sasaki M, Ozawa Y, Kurihara T, et al. Neuroprotective effect of an antioxidant, lutein, during retinal inflammation. *Invest Ophthalmol Vis Sci*. 2009;50:1433–1439.
21. Woo TT, Li SY, Lai WW, Wong D, Lo AC. Neuroprotective effects of lutein in a rat model of retinal detachment. *Graefes Arch Clin Exp Ophthalmol*. 2013;251:41–51.
22. Badaro E, Furlani B, Prazeres J, et al. Soluble lutein in combination with brilliant blue as a new dye for chromovitrectomy. *Graefes Arch Clin Exp Ophthalmol*. 2014;252: 1071–1078.
23. Caseli L, Sousa-Martins D, Maia M, Lima-Filho AA, Rodrigues EB, Belfort R Jr. An intraocular dye solution based on lutein and zeaxanthin in a surrogate internal limiting membrane model: a Langmuir monolayer study. *Colloids Surf B Biointerfaces*. 2013;107:124–129.
24. Furlani BA, Barroso L, Sousa-Martins D, et al. Lutein and zeaxanthin toxicity with and without brilliant blue in rabbits. *J Ocul Pharmacol Ther*. 2014;30:559–566.
25. Maia M, Furlani BA, Souza-Lima AA, Martins DS, Navarro RM, Belfort R Jr. Lutein: a new dye for chromovitrectomy. *Retina*. 2014;34:262–272.
26. Sousa-Martins D, Maia M, Moraes M, et al. Use of lutein and zeaxanthin alone or combined with Brilliant Blue to identify intraocular structures intraoperatively. *Retina*. 2012;32:1328–1336.
27. Vianna LMM, Cohen MJ, Muccioli C, et al. Efficacy of a lutein-based dye (PhacodyneTM) for visualizing anterior capsulorhexis during cataract surgery by phacoemulsification. *Arq Bras Oftalmol*. 2014;77:173–177.
28. Casaroli-Marano RP, Sousa-Martins D, Martinez-Conesa EM, et al. Dye solutions based on lutein and zeaxanthin: in vitro and in vivo analysis of ocular toxicity profiles. *Curr Eye Res*. 2014; 1–12.
29. Baszkin A. Molecular recognition on the supported and on the air/water interface-spread protein monolayers. *Adv Colloid Interface Sci*. 2006;128-130:111–120.
30. Brockman H. Lipid monolayers: why use half a membrane to characterize protein-membrane interactions? *Curr Opin Struct Biol*. 1999;9:438–443.
31. Dynarowicz-Latka P, Dhanabalan A, Oliveira ON Jr. Modern physicochemical research on Langmuir monolayers. *Adv Colloid Interface Sci*. 2001;91:221–293.
32. Krishnan V, Sakakibara K, Mori T, Hill JP, Ariga K. Manipulation of thin film assemblies: recent progress and novel concepts. *Curr Opin Colloid Interface Sci*. 2011;16:459–469.
33. Pavinatto FJ, Caseli L, Oliveira ON. Chitosan in nanostructured thin films. *Biomacromolecules*. 2010;11:1897–1908.
34. Morales MC, Freire V, Asumendi A, et al. Comparative effects of six intraocular vital dyes on retinal pigment epithelial cells. *Invest Ophthalmol Vis Sci*. 2010;51:6018–6029.
35. Yuen D, Gonder J, Proulx A, Liu H, Hutnik C. Comparison of the in vitro safety of intraocular dyes using two retinal cell lines: a focus on brilliant blue G and indocyanine green. *Am J Ophthalmol*. 2009;147:251–259, e252.
36. Fuller D, Machemer R, Knighton RW. Retinal damage produced by intraocular fiber optic light. *Am J Ophthalmol*. 1978;85: 519–537.
37. Kadonosono K, Takeuchi S, Yabuki K, Yamakawa T, Mekada A, Uchio E. Absorption of short wavelengths of endoillumination in indocyanine green solution: implications for internal limiting membrane removal. *Graefes Arch Clin Exp Ophthalmol*. 2003;241:284–286.

Investigative Ophthalmology & Visual Science

38. Narayanan R, Kenney MC, Kamjoo S, et al. Toxicity of indocyanine green (ICG) in combination with light on retinal pigment epithelial cells and neurosensory retinal cells. *Curr Eye Res*. 2005;30:471–478.
39. Cekic O, Chang S, Tseng JJ, Akar Y, Barile GR, Schiff WM. Cataract progression after intravitreal triamcinolone injection. *Am J Ophthalmol*. 2005;139:993–998.
40. Jonas JB, Degenring R, Vossmerbauemer U, Kamppeter B. Frequency of cataract surgery after intravitreal injection of high-dosage triamcinolone acetonide. *Eur J Ophthalmol*. 2005;15:462–464.
41. Jonas JB, Kreissig I, Degenring R. Intraocular pressure after intravitreal injection of triamcinolone acetonide. *Br J Ophthalmol*. 2003;87:24–27.
42. Marticorena J, Romano V, Gomez-Ulla F. Sterile endophthalmitis after intravitreal injections. *Mediators Inflamm*. 2012; 2012:928123.
43. Moshfeghi DM, Kaiser PK, Scott IU, et al. Acute endophthalmitis following intravitreal triamcinolone acetonide injection. *Am J Ophthalmol*. 2003;136:791–796.
44. Nelson ML, Tennant MT, Sivalingam A, Regillo CD, Belmont JB, Martidis A. Infectious and presumed noninfectious endophthalmitis after intravitreal triamcinolone acetonide injection. *Retina*. 2003;23:686–691.
45. Smithen LM, Ober MD, Maranan L, Spaide RF. Intravitreal triamcinolone acetonide and intraocular pressure. *Am J Ophthalmol*. 2004;138:740–743.
46. Jin Y, Uchida S, Yanagi Y, Aihara M, Araie M. Neurotoxic effects of trypan blue on rat retinal ganglion cells. *Exp Eye Res*. 2005; 81:395–400.
47. Luke C, Luke M, Dietlein TS, et al. Retinal tolerance to dyes. *Br J Ophthalmol*. 2005;89:1188–1191.
48. Narayanan R, Kenney MC, Kamjoo S, et al. Trypan blue: effect on retinal pigment epithelial and neurosensory retinal cells. *Invest Ophthalmol Vis Sci*. 2005;46:304–309.

Investigative Ophthalmology & Visual Science