Efficacy of Antibody Delivery to the Retina and Optic Nerve by Topical Administration

Stacy Hu¹ and Steven Koevary²

Abstract

Purpose: The purpose of this study was to determine whether nonspecific and ICAM-1-specific IgG1 antibodies can accumulate in the rat retina following topical application, and to develop a model system to show that antibodies that reach the posterior segment retain their pharmacological properties.

Methods: Eye drops containing mouse IgG1 or anti-ICAM-1 and the permeation enhancer saponin were topically applied to the eyes of Lewis rats. Concentrations were determined in the retina and optic nerve up to 30 min later using ELISA assays. We also developed an *in vitro* model to assess the pharmacologic activity of topically delivered antibodies in the retina based on the requirement of human umbilical vein endothelial cells (HUVECs) for vascular endothelial growth factor (VEGF) for growth. Rat eyes were treated with anti-VEGF antibody in the same manner as above; their retinas, harvested shortly thereafter, were added to HUVECs cultured in VEGF-containing media. The effect of these retinal homogenates on HUVEC proliferation was then assessed.

Results: Significant concentrations of IgG1 were detected in the optic nerve ($P < 0.001$) and retina ($P < 0.0001$) following topical application. Anti-ICAM-1 antibody also accumulated in the retina after topical application, though levels were less than those seen with IgG1 probably owing to a lower starting concentration. Retinal homogenates from eyes treated with anti-VEGF antibody significantly suppressed HUVEC proliferation ($P < 0.0001$).

Conclusion: Our data support the contention that topically applied antibodies can accumulate in the posterior segment, and suggest they retain their pharmacological properties.

Introduction

DUE TO THE CONSTRAINTS of the blood-aqueous and blood-ocular barriers, the targeting of therapeutic drugs to the posterior segment of the eye has best been accomplished by their local delivery to target tissues by either transscleral or intravitreal injection.^{1,2} On the other hand, the effectiveness of topical ocular delivery is limited by rapid drug elimination due to lacrimation and nasolacrimal drainage, drug binding to and metabolism by tear proteins, and target nonspecificity as a result of systemic absorption through the nasal and lacrimal duct mucosa and conjunctival vasculature.^{3,4} Despite these limitations, topically applied drugs reportedly accumulated in the posterior segment, and several topical therapies for retinal and choroidal diseases are under active development.^{5,6}

Over the past 15 years, several antivascular endothelial growth factor (anti-VEGF) compounds were developed that not only prevent the progression of wet macular degeneration but reverse its effects on visual acuity. To be effective, these compounds, such as the anti-VEGF antibody Avastin and its Fab fragment, Lucentis, must be administered by repeated intravitreal injections.^{7–13} Specifically, they are directly injected

into the vitreous cavity through the pars plana,¹⁴ thereby allowing high concentrations to reach their site of action. The requirement of repeated intravitreal injections to maintain treatment effectiveness has led to concerns regarding their ocular and systemic side effects. A small number of these patients developed endophthalmitis,^{15,16} while others developed immediate, transient elevations in intraocular pressure (IOP)^{17,18} or retinal pressure epithelium (RPE) tears and retinal detachments.^{19,20} A large review reported the incidence of retinal detachment to be as high as 0.9% per injection.²¹ Patients injected with Lucentis had a 2.1%–2.9% incidence of grade 3–4 ocular inflammation, while no age-related macular degeneration (AMD) patients receiving photodynamic therapy and/or sham injection had any cases of grade 3–4 ocular inflammation.^{16,22,23} Several trials evaluated whether less frequent or individualized dosing could reduce the risk of developing adverse events. While patients showed significantly less decline in visual acuity relative to those in the sham group, these end points were worse than in previous studies, suggesting that patients benefit from more frequent injections.²³

Thus, while anti-VEGF agents have been shown to be effective in the treatment of neovascular retinal diseases, the

¹Group Health Cooperative, Tacoma, Washington.

²New England College of Optometry, Boston, Massachusetts.

chronic need for their intravitreal injection to prevent further pathology and maintain treatment effectiveness was associated with a variety of side effects. In light of these data, and our previous findings suggesting that smaller topically applied proteins can accumulate in the retina, this study was designed to determine the efficacy of delivering antibodies to the posterior segment by topical application.

Methods

Animals

Two to 3-month-old female Lewis rats obtained from Harlan were used in this study. Rats were allowed access to food and water *ad libitum* in a climate-controlled room with a 12 h light/dark cycle. All experiments were performed according to the guidelines set by the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research, and were approved by the New England College of Optometry's Institutional Animal Care and Use Committee.

Topical application of IgG1

In our first series of experiments, a validated ELISA assay was used to quantify IgG1 levels in the retina following topical application. Initially, when we topically treated rats with a solution containing 1 mg/mL of mouse IgG1 antibody, we were unable to detect any IgG1 in the retina; accordingly, in subsequent experiments, we increased the concentration of our stock solution to 25 mg/mL.

To formulate the mouse IgG1 eye drop solution, 5 mg of IgG1 antibody (Mouse IgG1, catalog #M7894; Sigma, St. Louis, MO) were reconstituted in 200 μ L of sterile deionized water to create a final solution of 25 mg/mL. Serial dilutions of this reconstituted IgG antibody were also used to generate the ELISA standard curve. Each 10 μ L eye drop consisted of 9 μ L of the 25 mg/mL IgG1 solution, and 1 μ L of a 10% solution of the permeation enhancer saponin (Catalog #8047-15-2; Sigma) dissolved in sterile phosphate-buffered saline (PBS). Our previous studies with other peptides suggested the need for this permeation enhancer to facilitate movement through the ocular surface.^{24,25} Thus, the final concentration of IgG1 per drop was 22.5 mg/mL.

Forty-six female Lewis rats were used in this study. Based on our previous data, we speculated that IgG1 would most likely reach the retina and optic nerve in less than 30 min. Furthermore, it was most likely that optic nerve accumulation would be maximal at 10 min post-application, while retinal levels would likely peak 20 min post-application.^{24,26} Accordingly, in the first series of experiments, accumulation of IgG1 in the optic nerve was determined 10 min after topical application. Specifically, the right eyes of 10 rats received one 10 μ L drop prepared as above, while their left eyes served as untreated contralateral controls. Eight optic nerves from 4 untreated rats also served as negative controls. In the second series of experiments, accumulation of IgG1 in the retina was determined 20 min after topical application. Specifically, the right eyes of 20 rats received one 10 μ L drop prepared as above, while their left eyes served as untreated contralateral controls. Three additional rats received treatment in both their left and right eyes for a total of 6 treated retinas. Eighteen retinas from 9 untreated rats served as negative controls.

All animals, including those left untreated, were anesthetized with 0.3–0.8 mL of a ketamine/xylazine solution (2 parts 100 mg/mL ketamine:1 part 20 mg/mL xylazine) prior to eye drop application. Ten microliters of antibody solution were applied to the treated right eyes, after which they were closed and pressure applied for 5 min to prevent lacrimal drainage. In the first series of experiments in which optic nerve IgG1 levels were measured, the animals were sacrificed 10 min after treatment as mentioned above. The eyes were enucleated, leaving a several millimeter long segment of the optic nerves projecting from the posterior pole. From sacrifice, through the noting of their weights, to the placement of optic nerve segments into their respective 0.6 mL polypropylene tubes took less than 5 min on average. The tissues were then homogenized for at least 30 s on ice using a Kontes Pellet Pestle Motor with a 0.5 mL pestle (Kimble/Chase). Retinas were harvested in a similar manner, with the only difference being that animals were sacrificed 20 min after eye drop application. The tubes were spun down at 14,000 rpm for 3 min; supernatants were then harvested for use in the ELISA assay. The ELISA kit was purchased from Enzo Life Sciences (catalog #ADI-900-109; Enzo Biochem, Inc., Farmingdale, NY); standard curves were generated using IgG1 antibody diluted in retinal homogenates from untreated rats. Results were read at 450 nm using a BioTek EL-808 plate reader and were expressed as ng of mouse IgG1 per mg wet weight for the optic nerve data and per mg protein for the retina data, as measured using a BCA Protein Assay kit (Catalog #23225; Thermo Fisher Scientific, Waltham, MA). In all ELISA assays, the background was subtracted from all samples and standards.

Topical application of anti-ICAM-1 antibody

In addition to the polyclonal IgG1 antibody above, we also examined whether anti-ICAM-1 antibodies can accumulate in the retina after topical application; these antibodies were shown to inhibit inflammation when delivered by more direct means to the posterior segment^{27–30} by interfering with lymphocyte recruitment. Monoclonal anti-rat ICAM-1 antibody (500 μ g; anti-CD54, clone 141017, catalog #MAB5832; R&D Systems, Minneapolis, MN) was reconstituted in 50 μ L of sterile PBS to a final concentration of 10 mg/mL. Anti-ICAM-1 antibody eye drops were prepared with saponin in a manner similar to that described above. The right eyes of 10 anesthetized Lewis female rats were treated with an anti-ICAM-1 eye drop as described above for IgG1; the left eyes from the same animals served as untreated contralateral controls. Seven retinas from 4 untreated rats (1 retina sample was lost) served as untreated negative controls. Twenty minutes after treatment, the retinas were harvested as described for the IgG1 experiments, and retinal supernatants were assayed for their levels of anti-ICAM-1 antibody using the IgG1 ELISA; the anti-ICAM antibody used was also of the IgG1 subtype. Standard curves were generated using retinal homogenates spiked with anti-ICAM-1 antibody. The protein concentration in each sample was determined using the BCA protein assay as above, and the concentrations of anti-ICAM-1 antibody were adjusted accordingly.

Development of an in vitro HUVEC cell model to measure anti-VEGF activity

Rat eyes treated with anti-VEGF antibody were removed and their retinas harvested and homogenized. These retinal

homogenates were added into culture wells containing VEGF-containing media and human umbilical vein endothelial cells (HUVECs), which require VEGF for growth and replication. If anti-VEGF antibody had accumulated in the retina after topical application, then the addition of retinal supernatants containing these antibodies into the culture wells would be expected to negatively impact the growth of the HUVECs.

HUVEC cells

HUVECs (Cell line CRL-1730; ATCC, Manassas, VA) were passaged in F-12K Medium (Catalog #30-2004; ATCC). To make the complete growth medium, the following components were added: 0.1 mg/mL heparin, 0.03–0.05 mg/mL endothelial cell growth supplement (catalog #E-2759; Sigma), which consists of acidic and basic fibroblast growth factors, and fetal bovine serum (Catalog #10437-028; Thermo Fisher Scientific) to a final concentration of 10%.

VEGF bioassay

Confluent HUVECs were trypsinized with 0.5% trypsin-EDTA (Catalog #15400-054; Thermo Fisher Scientific), washed in F-12K basic media without growth factors (Catalog #21127-022; Thermo Fisher Scientific), and resuspended to a density of 100,000 cells/mL. Ninety microliters of this cell suspension were added to the wells of a Corning 96-well tissue culture plate so that the final density of cells in each well was $\sim 10^4$ cells/well. In a preliminary experiment, serial dilutions of recombinant human VEGF165 (Catalog #293-VE-010; R&D Systems) ranging from 100 ng/mL to 1.5 ng/mL were added to the wells (8 wells/dilution). This was carried out to provide information regarding the best concentration of VEGF to use in subsequent assays. Cells cultured in the absence of VEGF served as negative controls.

In a separate assay, HUVECs were incubated as above in basic media and 100 ng/mL VEGF, which was deemed ideal for growth as determined in the previous assay. To determine how much anti-VEGF was needed to suppress growth, cells were incubated with anti-VEGF antibody (Catalog #AF-293-NA; R&D Systems) at concentrations of 10, 1, or 0.5 mg/mL in groups of 8 wells each. Cells grown in VEGF-containing media without anti-VEGF antibody served as positive controls.

In the final assay of this series, retinal homogenates from eyes previously treated with anti-VEGF antibody were tested for their ability to suppress VEGF-induced HUVEC growth, and results were compared to the antibody assay above. Ten female Lewis rats were used for this study; 4 right eyes from 4 rats served as untreated controls, while 6 right eyes from each of 6 rats were treated topically with anti-VEGF antibody. Three of these 6 rats were sacrificed and their eyes harvested after 10 min post-treatment, while the remaining 3 rats were sacrificed after 20 min post-treatment.

The anti-VEGF antibody solution was formulated as follows: a 200 μ g stock bottle of anti-VEGF antibody (same as above) was diluted with 108 μ L of sterile PBS and 12 μ L of sterile 10% saponin (1% final solution) to achieve a final volume of 120 μ L. All animals were anesthetized as above prior to eye drop administration. Ten microliters of this solution, which contained 166 μ g of the antibody, were applied to the right eye of each treated animal, after which the eyes were closed and pressure applied for a full 10 min; pressure was applied for twice the amount of time as in the experiments

above because of the particularly low concentration of our starting stock solution. Three rats were sacrificed 10 min after eye drop application and the remaining 3 were sacrificed 20 min after application. The retinas were harvested, weighed, and placed into 30 μ L of sterile PBS in sterile 0.6 mL polypropylene microfuge tubes on ice. They were then homogenized on ice and the homogenates spun down in a microfuge for 3 min at 14,000 rpm. The homogenates were transferred to empty microfuge tubes and respun for an additional minute to enhance their clarity. Ten microliters of each of these homogenates were added into the assay wells in duplicate; as above, the assay wells contained just under 10^4 cells/well and 100 ng/mL of VEGF.

The plates in all of the above assays were incubated for 48 h at 37°C in 5% CO₂ in air, after which most of the media were aspirated from the wells and 100 μ L of phenol red-free DMEM media (Catalog #A14430-01; Thermo Fisher Scientific) were added back into the wells. This step was necessitated by the fact that phenol red interferes with the Vibrant MTT Cell Proliferation Assay Kit (Catalog #V13154; Thermo Fisher Scientific) that was used to quantify cell proliferation. Specifically, 10 μ L of a 12 mM MTT stock solution were added to each well and the plates incubated for an additional 4 h, after which all but 25 μ L of media in each well were aspirated and 50 μ L of DMSO (Catalog #20688; Thermo Fisher Scientific) added to each well to dissolve the cells. The plates were then briefly shaken on a plate shaker and incubated for 10 min at 37°C, after which the plates were again briefly shaken and the absorbance in the wells determined at 540 nm using the BioTek EL-808 plate reader. Results were expressed as absorption units per mg tissue. Some cultures were photographed under phase microscopy prior to the initiation of the MTT assay.

Statistical analysis

All statistical analyses were conducted using JMP and Statistical Software from SAS, and graphs were created using Excel or SigmaPlot 12.0. Data in the graphs are expressed as the mean $\pm$ standard error.

To determine whether nonparametric statistical analyses were needed, all data were subjected to tests for normality. The data were analyzed using either Student's *t*-tests or one-way ANOVAs with *post hoc* comparisons when indicated through Tukey's honest significant difference (HSD) test assuming normal distributions. Non-normal data were analyzed via Kruskal–Wallis with pair-wise *post hoc* testing conducted using the Mann–Whitney test when indicated. All probability values were 2-tailed. $P < 0.05$ was considered to be significant.

Results

Topically applied mouse IgG1 antibodies accumulated in the optic nerve and retina

Statistically significant levels of IgG1 were detected in the optic nerves in both the treated and contralateral, untreated eyes of rats 10 min posteye drop application compared with controls ($P < 0.001$; Fig. 1). IgG1 levels in optic nerve homogenates from treated eyes were 4.92 ± 2.08 ng/mg tissue, compared to 0.06 ± 0.06 ng IgG1 per mg tissue in control eyes ($P < 0.001$). Interestingly, and in concert with our previous studies,²⁴ untreated contralateral eyes within the same animal

FIG. 1. Levels of IgG1 in the optic nerves of treated and untreated, contralateral eyes were significantly higher than controls. IgG1 levels in treated and contralateral eyes were also significantly different from each other ($P < 0.05$). * $P < 0.001$ and ** $P < 0.01$ compared to untreated controls. Data are expressed in ng IgG1 antibody per mg optic nerve wet weight ($n = 10$ treated, 10 contralaterals, 8 controls).

demonstrated increased levels of IgG1 compared with controls (0.84 ± 0.31 ng IgG1 per mg, $P < 0.01$), albeit at significantly lower values compared with treated eyes ($P < 0.05$); this observation will be addressed in the Discussion section.

Similarly, statistically significant levels of IgG1 were detected in the retinas in both treated and contralateral untreated eyes 20 min posteye drop application ($P < 0.0001$, Fig. 2) compared with controls. IgG1 levels in retinal homogenates from treated eyes were 8.07 ± 1.72 ng IgG1 per mg protein, compared to 0.17 ± 0.10 ng IgG1 per mg protein in untreated control eyes ($P < 0.0001$). Untreated contralateral eyes within the same animal also demonstrated increased levels of IgG1

FIG. 2. Levels of IgG1 in the retinas of treated and untreated, contralateral eyes were significantly higher than controls. IgG1 levels in treated and contralateral eyes were also significantly different from each other ($P < 0.01$). * $P < 0.0001$ and ** $P < 0.001$ compared to control. Data are expressed in ng IgG1 antibody per mg retinal wet weight ($n = 26$ treated, 20 contralaterals, 18 controls).

compared with controls (1.83 ± 0.65 ng IgG1 per mg protein, $P < 0.001$). Again, levels of IgG1 in treated eyes were higher than in contralateral eyes ($P < 0.01$).

Topically applied anti-ICAM-1 antibodies accumulated in the retina

Anti-ICAM-1 antibody accumulated in the retina 20 min after topical application, though levels were less than those seen with IgG1. Anti-ICAM-1 antibody levels in retinal homogenates from treated eyes were 1.90 ± 0.64 ng IgG1 per mg protein; no anti-ICAM-1 antibody was detectable in controls ($P < 0.05$, Fig. 3). Unlike in our IgG1 experiments, there was no difference in the concentrations of anti-ICAM-1 antibody levels between the untreated contralateral eyes and controls (contralateral: 0.89 ± 0.36 ng IgG1 per mg protein).

VEGF bioassay

VEGF had a dose-dependent effect on HUVEC cell growth (Fig. 4). Statistical analysis revealed a significant reduction in cell growth between cultures incubated with a dose of 100 ng/mL compared with all other doses below 25 ng/mL ($P < 0.001$). Fig. 5 shows phase contrast microscope photographs of HUVECs cultured in high or low concentrations of VEGF. In the relative absence of VEGF, cells appeared clumped, detached, and non-viable, while the cells cultured with VEGF appeared healthy and were attached. Based on the results of this assay, we used VEGF at a concentration of 100 ng/mL in all subsequent assays.

The addition of anti-VEGF antibody to cultured HUVECs significantly suppressed their growth in a dose-dependent fashion (Fig. 6). HUVECs cultured in the absence of VEGF served as negative controls, and their growth was also significantly reduced compared to cultures that contained VEGF. Retinal homogenates from the eyes of rats treated with anti-VEGF antibody 10 or 20 min earlier significantly suppressed HUVEC proliferation, as determined by the MTT assay (Fig. 7; $P < 0.0001$). Though both time points

FIG. 3. Levels of anti-ICAM-1 were significantly higher in the retinas of treated eyes compared with controls. There was no difference between antibody levels in contralateral and control eyes. * $P < 0.05$ compared to untreated controls. Data are expressed in ng anti-ICAM-1 antibody per mg retinal protein ($n = 10$ treated, 10 contralaterals, 7 controls).

FIG. 4. Human umbilical vein endothelial cells (HUVEC) cell growth was dependent on vascular endothelial growth factor (VEGF) in a dose-dependent manner.

were significantly different than controls, the proliferation nadir appeared to be closer to 10 min, since the 20 min values were also significantly higher than those at the earlier time point ($P < 0.001$).

FIG. 5. Phase contrast microscopy of HUVECs cultured in (A) high (100 ng/mL) and (B) low (1.25 ng/mL) concentrations of VEGF. Note the detached, clumped dead cells (B, arrows) compared to the healthy, attached cells in (A).

Suppression of HUVEC Cell Growth by Anti-VEGF Antibody

FIG. 6. Anti-VEGF antibody added *in vitro* inhibited HUVEC growth in a dose-dependent manner. * $P < 0.01$ compared to VEGF alone.

Discussion

Our data support the hypothesis that full-sized antibodies, applied topically together with a permeation enhancer, can accumulate in the rat optic nerve and retina. Specifically, polyclonal IgG1 and anti-ICAM-1 antibodies, the latter of which have been used to suppress ocular inflammation, were detectable in the posterior pole less than 30 min after topical application. This time course is consistent with our previous data involving the use of insulin and glial-derived neurotrophic factor.^{24,26,31}

Interestingly, the contralateral eyes also accumulated both IgG1 and anti-ICAM-1 following topical application, a phenomenon that we and others have reported with other peptides. Intravitreal and subconjunctival injection of antibody in rabbits both resulted in accumulation in the untreated, fellow eye;

Effects of Retinal Homogenates Derived from Rats Treated with Anti-VEGF Antibody on HUVEC Cell Growth

FIG. 7. Retinal homogenates from eyes treated with anti-VEGF antibody inhibited proliferation of HUVECs *in vitro*. Animals were sacrificed either 10 or 20 min after topical anti-VEGF antibody application. * $P < 0.001$ compared to control. Data are expressed as absorbance per mg retinal wet weight ($n = 6$ treated [3 at 10' postapplication, 3 at 20' postapplication], 6 contralaterals, 4 controls).

in those studies, the authors speculated that this might be due to uptake following systemic absorption.³² Our previous data, however, showed that contralateral uptake even occurred in decapitated animals, indicating that systemic uptake was not the mechanism.^{24,33} In light of our earlier findings of high levels of topically applied peptides in the cerebrospinal fluid (CSF), we previously speculated that these peptides reached the posterior pole of the contralateral eye by diffusing through the CSF that surrounds the optic nerve and chiasm. Our previous data also suggested that while systemic uptake following topical application does occur, it does not influence the levels of peptide recovered in retinal homogenates, nor did it appear in under 30 min²⁵; recall that our working time frame was less than 30 min postapplication. In this study, we were not able to recover measurable amounts of IgG1 from serum at 10 and 20 min after application, though we were able to measure IgG1 in serum that was spiked with antibody (data not shown).

We utilized an *in vitro* bioassay model to examine whether antibodies that accumulated in the retina following topical application retain their ability to effect pharmacological changes. Specifically, the model was based on the need for VEGF for the growth of HUVECs. Our data showed that retinal homogenates from anti-VEGF-treated rat eyes suppressed cell growth and proliferation, supporting the notion that the topically delivered antibodies mediated this effect. It is important to note that this bioassay only indirectly suggested the presence of anti-VEGF antibody in the retinal homogenates. Further experiments will need to be carried out to confirm the presence and activity of this antibody *in vivo*.

Our results showing that topically applied antibodies accumulate in the posterior pole go against the conventional wisdom that suggests molecules larger than 40 kDa are not able to reach the retina and optic nerve in detectable levels.^{32,34–36} That being said, there have been other reports of antibody movement to the posterior pole following topical application. Chen et al. showed that topically delivered Lucentis, the 48 kDa antibody fragment of the parent anti-VEGF antibody Avastin, was able to reach the retina in rabbits. However, their sample sizes were small, and not all of their animals had detectable antibody levels in these tissues. Furthermore, the authors used confocal immunohistochemistry to localize the antibody and did not take quantitative measurements.³⁷

Nomoto et al. compared the pharmacokinetics of Avastin after topical delivery, subconjunctival injection, and intravitreal injection in rabbits. The authors found that intravitreal injections resulted in the highest concentrations of the antibody in the retina, and they also found detectable levels in the retina/choroid in both treated and contralateral untreated eyes following topical application at all time points tested. The authors did not state whether these levels were significantly different between the 2 eyes, or whether they were significantly higher than controls. As in the study by Chen et al. the authors did not evaluate antibody levels in the retina at the same time points as we did in this study. Finally, the authors did not examine whether retinal Avastin was able to inhibit the activity of VEGF. Our experiments quantified the levels of antibody that accumulated in the retina following topical application, and provided suggestive evidence that the antibodies that accumulate there may retain their pharmacological activity.

While our data support the contention that topically applied antibodies can reach the retina, levels detected there were several orders of magnitude lower than the concentration of the solution that was applied to the ocular surface.

Similar reductions were reported following topical application of antibodies in the rabbit.³⁸ Thus, high concentrations would have to be applied to get potentially therapeutic levels into the retina; interestingly, our bioassay results suggest this can be achieved, since the concentrations of antibody that we applied to the surface of rat eyes were similar to the concentrations of stock solutions of Avastin and Lucentis used for the treatment in humans.⁷ Another potential advantage of topical administration of anti-VEGF is its decreased accumulation in the systemic circulation. Nomoto et al. showed that topical administration of bevacizumab 6 times a day for 7 days resulted in detectable levels of the antibody in the plasma 1 week later, but others reported that systemic concentrations of a much smaller antibody fragment ESBA105 (a 26 kDa anti-TNF antibody) was 25,000 times lower after topical administration compared to systemic concentrations after intravitreal injection.³⁹

Intravitreal injection of anti-VEGF agents has a well-established association with systemic adverse events. The injected agents were quickly detected in the blood stream 1 day after the first dose, and resulted in a marked reduction in VEGF in the plasma that persisted for as long as 28 days.^{40,41} Likely as a result of their detectable inhibitory effect on plasma-free VEGF, intravitreally injected anti-VEGF antibodies were reportedly associated with an increased risk of systemic adverse events such as myocardial infarction, cerebrovascular accidents, transient ischemic attacks, deep vein thrombosis, pulmonary embolism, and death.^{23,42–44} A Cochrane review found that 139 per 1,000 patients treated with Lucentis experienced at least 1 systemic adverse event after 1 year, compared with 177 per 1,000 patients treated with Avastin; the difference in systemic safety profiles between the 2 drugs was not significant.^{11,13} Thulliez et al. concluded that intravitreal anti-VEGF antibodies did not result in statistically significant increases in major cardiovascular or systemic hemorrhages, but agreed that “studies and meta-analyses were not powered enough to correctly assess these risks”.⁴⁵ Thus, caution is still urged for patients receiving intravitreal injections of anti-VEGF antibodies who are especially at risk for systemic adverse events.

While data collected from all of our previous studies suggest only minimal systemic absorption following topical application, an extensive time course of antibody uptake in the bloodstream was not investigated. It is possible that over time, antibody levels would rise in the blood. This could result in systemic consequences that would need to be addressed if this approach is to be seriously investigated as an alternative to intravitreal injection.

In summary, our data suggest that topically delivered full-sized antibodies can accumulate in the retina and optic nerve, and provide suggestive evidence based on our *in vitro* model that they retain their pharmacological activity. Multiple hurdles remain that must be overcome to advance the field of topical drug delivery for the treatment of posterior segment disease; notably, the marked drop in the concentration of drug that reaches the retina and the potential for significant systemic uptake should multiple weekly treatments be required.

Author Disclosure Statement

No competing financial interests exist.

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Address correspondence to:

Dr. Steven Koevary
New England College of Optometry
424 Beacon Street
Boston, MA 02115

E-mail: koevarys@neco.edu