

Trans-scleral Delivery of Antiangiogenic Proteins

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ABSTRACT

Purpose: In this study, we investigated the penetration of various proteins into the mouse eye after a periocular injection of the protein or an adenoviral vector (Ad) expressing the protein.

Methods: At several time points after the injection, the retina, retinal pigmented epithelium/choroid, and sclera were dissected and enzyme-linked immunosorbent assays were performed.

Results: After a periocular injection of AdsFlt-1.10, AdTGF β .10, or AdPEDF.11, choroidal levels of pigment epithelium-derived factor (PEDF) and transforming growth factor- β (TGF- β) were not significantly different from scleral levels, and choroidal levels of sFlt-1 (soluble Flt-1 or soluble VEGF receptor 1) were only moderately reduced from scleral levels, indicating that each of these proteins penetrate the sclera well. In contrast, retinal levels of each of the three proteins were low compared to choroidal levels, suggesting poor penetration into the retina. Levels of PEDF in the choroid peaked 2 h after a periocular injection of PEDF protein and returned to baseline between 6 and 24 h, and peak levels in the retina were 8.6% of peak choroidal levels. Levels of green fluorescent protein, a protein unlikely to have any binding sites in mouse tissues, peaked in the choroid 2 h after the periocular injection and were undetectable by 4 h, while peak levels in the retina were 64.3% of peak choroidal levels.

Conclusions: These data suggest that size and binding characteristics of proteins are likely to influence their ability to penetrate the eye from the periocular space, but in general, proteins as large as 50–75 kDa penetrate well into the choroid, but not into the retina. Periocular injections are feasible for the treatment of choroidal neovascularization with proteins or vectors that express them, but additional investigations are needed before they can be considered for treatment of retinal diseases.

INTRODUCTION

ELUCIDATION OF the molecular pathogenesis of a disease process provides targets for therapeutic intervention. Several lines of evidence im-

plicated vascular endothelial growth factor (VEGF) as a critical stimulus for both retinal and choroidal neovascularization,^{1–6} which prompted the development of VEGF antagonists. Proof of concept has been provided by clinical trials that

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showed that repeated intravitreal injections of ranibizumab, a Fab fragment that binds all isoforms of VEGF-A, results in significant improvement in vision in roughly 30%–40% of patients with neovascular age-related macular degeneration (NVAMD).^{7,8} Intraocular injections of ranibizumab are now standard care for patients with NVAMD and are being investigated in clinical trials for several other retinal diseases.⁹

Intravitreal injections allow for a rapid delivery of agents to the retina and choroid but carry the risk of endophthalmitis and retinal detachment. Also, they result in high peak levels of drug separated by troughs that may be below the therapeutic threshold, which may be fine for some but not all agents. Less invasive routes of delivery that achieve sustained therapeutic drug levels could provide major benefits. Delivery from the periocular space is appealing, because it does not require needle penetration into the eye. Recently, it has been shown that it is possible to inhibit choroidal neovascularization by expressing pigment epithelium-derived factor (PEDF) or soluble VEGF receptor 1 (sFlt-1) on the outside of the eye in mice or pigs.^{10–12} This indicates that proteins as large as 50 kd (PEDF) or 75 kd (sFlt-1) are able to penetrate the sclera and suprachoroidal space to reach the site of choroidal neovascularization. In this study, we sought to obtain a quantitative assessment of protein delivery to intraocular tissues from the periocular space.

METHODS

Adenoviral (Ad) vectors

Production and quantification of type 5 adenoviral vectors that express β -galactosidase (LacZ), human PEDF, sFlt-1, or TGF- β 1 from a cytomegalovirus (CMV) immediate early promoter expression cassette have been previously described.^{11,13–15} The vectors were deleted for E1A, E1B, and E3 and either contained the intact E4 region (AdLacZ.10, AdsFlt1.10, and AdTGF β 1.10) or were deleted for the E4 region (AdPEDF.11).

Measurements of the thickness of the sclera, choroid/RPE, and retina in mice

Adult (6–8 week old) female C57BL6 mice (Jackson Laboratories, Bar Harbor, ME) were

treated in accordance with the ARVO statement for the use of animals in ophthalmic and vision research. The mice were euthanized, eyes were frozen in optimal cutting temperature embedding medium (Miles Diagnostics, Elkhart, IN), and serial 10- μ m frozen sections were cut from one edge of the optic nerve to the other. The sections were fixed in 4% paraformaldehyde for 30 min at 4°C, rehydrated in deionized water for 30 sec, stained with hematoxylin and eosin, and mounted in Cytoseal (Fischer Scientific, Pittsburgh, PA). Ten (10) slides per eye were examined with a Zeiss Axioskop microscope (Zeiss, Thornwood, NY), and the mean thickness of the sclera, choroid/RPE, and retina was measured adjacent to the optic nerve, halfway between the optic nerve and the equator, and at the equator by image analysis with Image Pro Plus (Media Cybernetics, Silver Spring, MD) to give a single experimental value. Measurements were made in 5 mice.

Periocular injections

Adult C57Bl/6 mice were anesthetized by a subcutaneous injection of 50 mg/kg of ketamine hydrochloride (Schein Pharmaceuticals, Port Washington, NY), and periocular injections were done with a disposable 32-gauge needle on a Hamilton syringe (Hamilton, Reno, Nevada), as previously described.^{10,11} The conjunctiva was grasped and elevated on the temporal side of the globe 3 mm posterior to the limbus taking care to avoid hemorrhage from damage to a conjunctival blood vessel. The needle was inserted through the elevated conjunctiva and advanced 3–4 mm and the 3-, 5-, or 7- μ L volume in the syringe was slowly injected, resulting in a bleb that was highest temporally and gradually spread around the globe.

Intravitreal injections

Intraocular injections were performed by using a Harvard pump microinjection apparatus (Harvard Apparatus, Holliston, MA) and pulled glass micropipets, as previously described.¹⁴ Each micropipet was calibrated to deliver 1 μ L containing 10⁹ vector particles upon depression of a foot switch. The mice were anesthetized, pupils were dilated, and under a dissecting microscope the sharpened tip of the micropipet was passed through the sclera just behind the limbus into the vitreous cavity and the foot switch was depressed.

Assessing effect of injection volume on scleral distribution

BALB/c mice (Jackson Laboratories) were given a periocular injection of 3, 5, or 7 μL of Higgins Black waterproof ink (Eberhard Faber, Inc., Lewisburg, TN) and euthanized after 1 h. Scleral flat mounts were prepared, examined with an Axioskop microscope, and the total and ink-stained areas of sclera were measured by image analysis using Image Pro Plus. In similar experiments, BALB/c mice were given a periocular injection of 3, 5, or 7 μL of AdLacZ.10 (10^9 particles/ μL). Twenty-four (24) h after injection, the mice were euthanized, the eyes were removed, fixed in 0.5% glutaraldehyde in phosphate-buffered saline (PBS) for 1 h, rinsed twice in 25% sucrose in PBS, and incubated overnight in 1 mg/mL of X-gal (5-bromo-4-chloro-3-indolyl galactopyranoside; Sigma, St. Louis, MO) in a solution containing 5 mM $\text{K}_3\text{Fe}(\text{CN})_6$, 5 mM $\text{K}_4\text{Fe}(\text{CN})_6 \cdot 3\text{H}_2\text{O}$, and 1 mM MgCl_2 in PBS. The eyes were postfixed for 15 min in 0.5% glutaraldehyde in PBS and washed in PBS. Scleral flat mounts were prepared, examined with an Axioskop microscope, and the total and Xgal-stained areas of sclera were measured by image analysis.

Measurement of intraocular protein levels after intraocular or periocular gene transfer

Adult C57BL/6 mice were given a periocular injection of 3, 5, or 7 μL of AdsFlt1.10 (10^9 particles/ μL) and 48 h after injection, the mice were anesthetized, and blood was removed by a cardiac puncture. The blood was centrifuged and serum was stored at -80°C . The mice were euthanized and the vector-injected eye and the uninjected contralateral eye were removed and the sclera, RPE/choroid, and retina were dissected and snap frozen. The tissues were mechanically homogenized with a mortar and pestle, sonicated in PBS/100 μM PMSF, using a Branson 2510 sonicator (Branson, Danbury, CT), and microfuged. Total protein was measured in supernatants, using the Bio-Rad Protein Assay (Bio-Rad, Hercules, CA), and concentrations of sFlt-1 were measured using an enzyme-linked immunosorbent assay (ELISA) kit for sFlt-1 (R&D systems, Minneapolis, MN).

Adult mice were given an intraocular injection of 1 μL or a periocular injection of 5 μL of AdPEDF.11 or AdTGF β .10 (each 10^9 particles/ μL) and after 48 h mice were euthanized, the eyes were removed, and the sclera, RPE/choroid, and retina were dissected. The tissues were homogenized as described

above, total protein was measured the Bio-Rad Protein Assay, and the concentration of PEDF was measured by ELISA, as previously described, and the concentration of TGF β -1 was measured with an ELISA kit (R&D systems). The limit of sensitivity of the assay was 0.1 ng/mL.

Measurement of PEDF levels at several times after periocular injection of human recombinant PEDF

Human recombinant PEDF was prepared, as previously described.¹⁶ Adult C57BL/6 mice were given a periocular injection of 5 μL containing 3.65 μg of PEDF in one eye and no injection in the contralateral eye. At 0.17, 0.34, 1, 2, 6, and 24 h after the injection, mice were euthanized, eyes were removed, and the sclera, RPE/choroid, and retina were dissected. Levels of PEDF were measured as described above.

Measurement of GFP levels at several times after periocular injection of GFP

Mice were given a received a periocular injection of 5 μL containing 3.65 μg of recombinant GFP (Roche Applied Science, Indianapolis, IN) in one eye and no injection in the contralateral eye. At 0.17, 0.34, 0.5, 1, 2, and 4 h after the injection, mice were euthanized, the eyes were removed, and the sclera, choroid, and retina were dissected. Tissue homogenates were prepared and total protein was measured, as described above, and concentrations of GFP were measured, using a two-antibody sandwich ELISA in 96-well Reacti-bind Anti-GFP immunoplates (Pierce, Rockford, IL). A serial dilution of recombinant GFP was used to generate a standard curve.

Statistical analyses

Statistical comparisons were made by analysis of variance (ANOVA) with Dunnett's correction for multiple comparisons, using SAS software (SAS Institute, Inc., Cary, NC). For ELISA data, a log transformation was utilized to normalize the distribution prior to analysis.

RESULTS

Scleral and choroidal thickness in mouse eyes

Mouse eyes were snap frozen and scleral and choroidal thickness was measured on frozen sec-

tions at several locations. Scleral thickness was $9.7 \pm 0.36 \mu\text{m}$ at the equator and $20.1 \pm 0.69 \mu\text{m}$ adjacent to the optic nerve, and choroidal thickness was $14.4 \pm 0.33 \mu\text{m}$ at the equator and $19.4 \pm 0.66 \mu\text{m}$ adjacent to the optic nerve.

Effect of injection volume on scleral exposure in mouse eyes

One (1) h after the periocular injection of 3, 5, or 7 μL of ink, scleral flat mounts showed that a

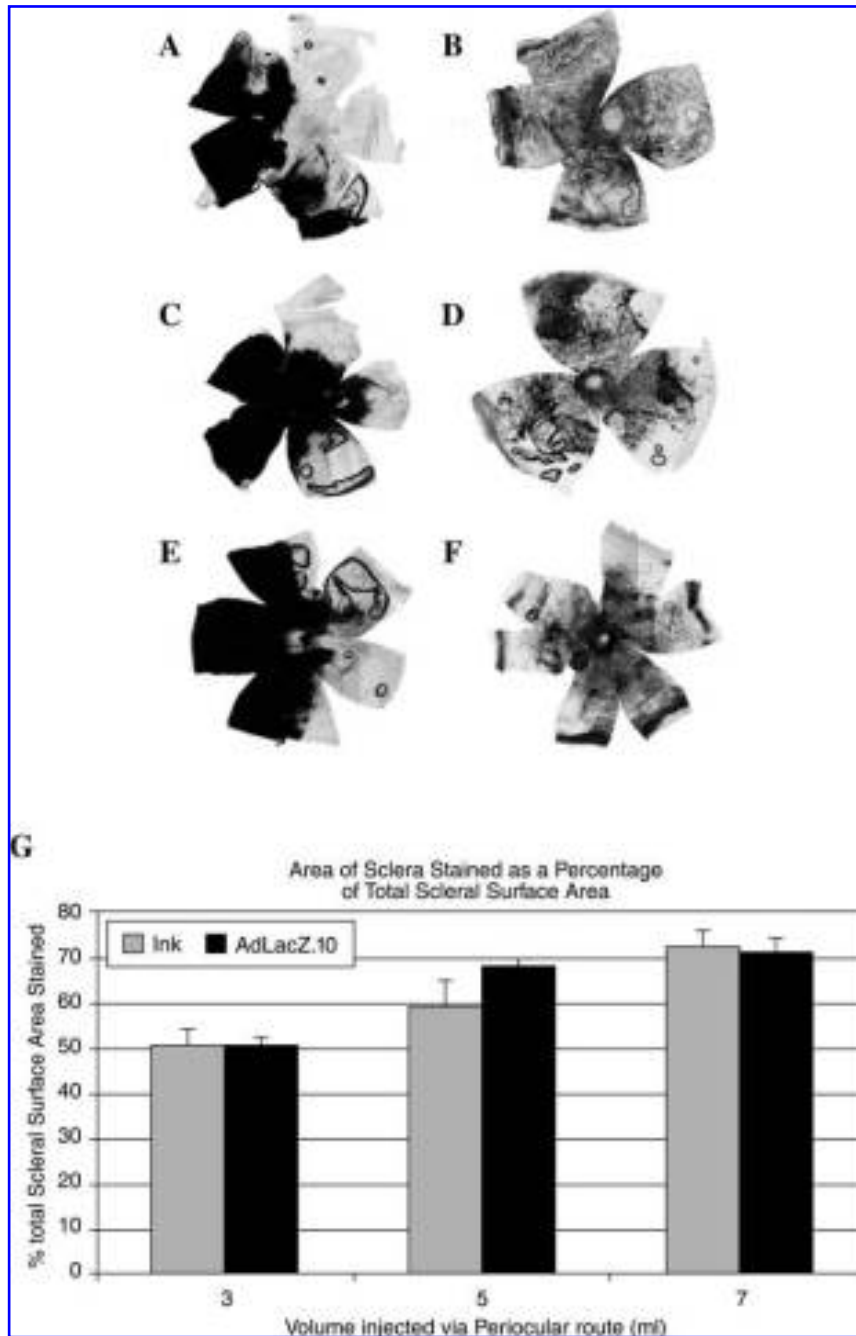


FIG. 1. Effect of injection volume on scleral exposure in mouse eyes. Mice were given a periocular injection of 3, 5, or 7 μL of ink and scleral flat mounts were prepared 1 h after injection. Forty-eight (48) h after a periocular injection of 3, 5, or 7 μL of AdLacZ.10 (10^9 particles/ μL), eyes were stained with Xgal and scleral flat mounts were prepared. For each injection volume, the area of sclera stained with ink or transduced with AdLacZ.10 appeared similar and there appeared to be only modest differences between eyes injected with 3 μL (A, B), 5 μL (C, D), or 7 μL (E, F). Image analysis confirmed that there were no significant differences between the areas of ink-stained sclera and transduced sclera for each volume injected (G). Each bar represents the mean (\pm standard error of the mean) calculated from five experimental values.

substantial portion of the sclera was stained with ink for each of the injection volumes (Fig. 1A, 1C, and 1E). Forty-eight (48) h after the injection of 3, 5, or 7 μL of AdLacZ.10 (10^9 viral particles/ μL), the Xgal-stained area of the sclera (Fig. 1B, 1D, and 1F) appeared quite similar to the ink-stained area for each injection volume. Image analysis confirmed that there were no significant differences between the area of sclera stained with ink and the area transduced by AdLacZ.10 for each periocular injection volume (Fig. 1G). These data show that a substantial portion of the sclera is exposed to solutions injected into the periocular space, with only modest increases when the injection volume is increased from 3 to 7 μL .

Intraocular levels of sFlt-1 after periocular injection of AdsFlt1.10

Periocular injection of AdsFlt1.10 strongly suppresses choroidal neovascularization.¹¹ To determine the intraocular levels of sFlt-1 that are responsible for this therapeutic effect, we did periocular injections of 3, 5, or 7 μL of AdsFlt1.10 (10^9 viral particles/ μL). The mean level of sFlt-1

in the choroid was 0.78 $\text{pg}/\mu\text{g}$ total protein after the periocular injection of 5 μL of AdsFlt1.10 and was not statistically different after an injection of 3 or 7 μL (Fig. 2). Compared to the level of sFlt-1 achieved in the choroid after a periocular injection of 5 μL of AdsFlt1.10, the mean level of sFlt-1 in the retina was substantially less, 0.14 $\text{pg}/\mu\text{g}$ total protein. Somewhat surprisingly, this was not significantly different from the level of 0.11 $\text{pg}/\mu\text{g}$ total protein achieved in the retina after an intraocular injection of 10^9 particles of AdsFlt1.10. However, significantly higher levels of sFlt-1 occurred in the cornea after an intraocular injection of 10^9 particles of AdsFlt1.10, compared to a periocular injection of any of the three concentrations tested. This is consistent with previous studies that showed that an intraocular injection of adenoviral vectors results in prominent transduction of the cornea, with little transduction of the retina.^{14,15} Levels of sFlt-1 were not increased above baseline in the serum or the contralateral choroid after a periocular injection of AdsFlt1.10, indicating that the levels of sFlt-1 observed in the injected eye were due to the penetration through the sclera and not from the systemic circulation.

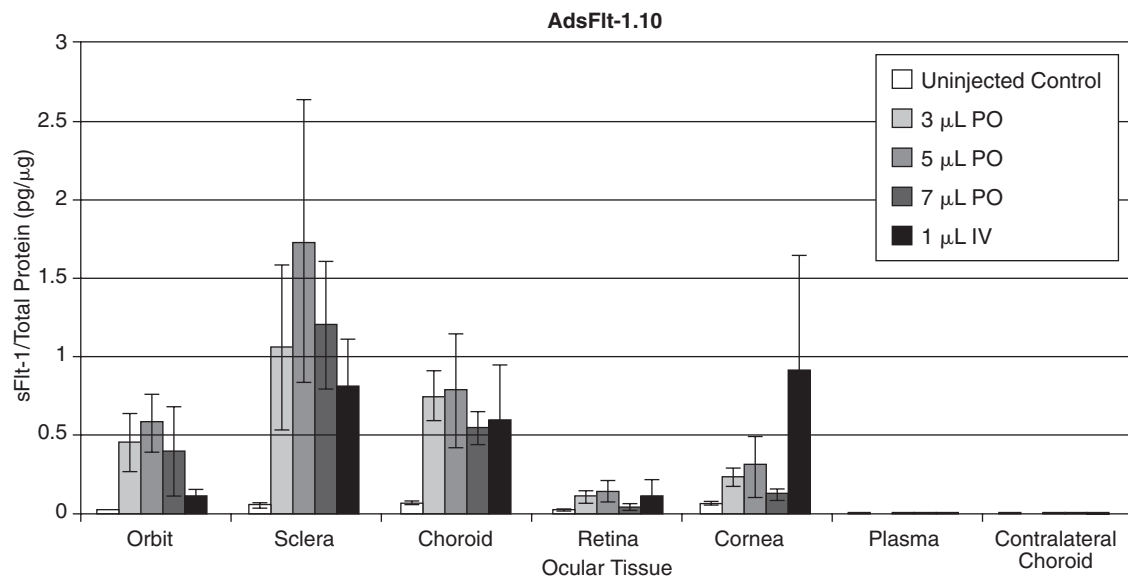


FIG. 2. Levels of sFlt-1 in ocular tissues or serum after periocular or intraocular injection of different amounts of AdsFlt1.10. Forty-eight (48) h after a periocular injection of 3, 5, or 7 μL or an intravitreal injection of 1 μL of AdsFlt1.10 (10^9 viral particles/ μL), the level of sFlt-1 was measured in ocular tissues or serum. Each bar represents the mean (\pm standard error of the mean) calculated from six experimental values. There were no significant differences between levels obtained in each tissue from a periocular injection of 3 or 5 μL , with significantly lower levels in retina and choroid following a 7 μL of AdsFlt1.10. Levels of sFlt-1 were significantly less in retina, compared to choroid for all injection volumes and routes. The level of sFlt-1 was significantly greater ($P < 0.05$) in the cornea after an intraocular injection, compared to a periocular injection of any of the three volumes of AdsFlt1.10. The levels of sFlt-1 in serum or contralateral choroid were not significantly greater than the uninjected controls. Statistical comparisons were made by analysis of variance with Dunnett's correction for multiple comparisons. PO = Periocular; IV = intravitreal.

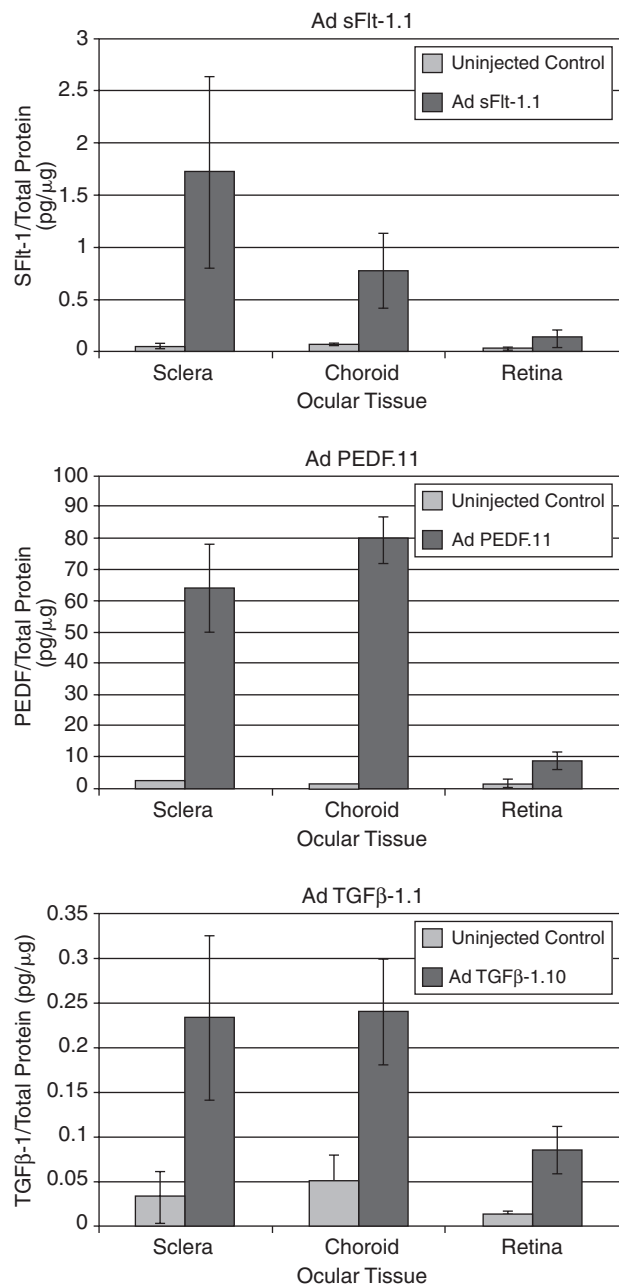
Intraocular levels of PEDF or TGF- β after periocular injection of AdSPEDF.11 or AdTGF β 1.10

Periocular injection of AdPEDF strongly suppresses choroidal neovascularization.¹⁰ Compared to the levels of sFlt-1 obtained in the sclera after the periocular injection of 5 μ L containing 5×10^9 viral particles of AdSPEDF.11 (Fig. 3A), the level of PEDF in the sclera after a periocular injection of 5 μ L containing 5×10^9 viral particles of AdPEDF.11, were substantially higher (Fig. 3B). It was also higher than the level of TGF β 1 in the sclera after a periocular injection of 5 μ L containing 5×10^9 viral particles of Ad TGF β 1.10 (Fig. 3C). This suggests that the transduction and/or translation efficiency is greater for AdPEDF.11 than the other two vectors. Regardless of the different levels in sclera, the penetration of each of the three proteins through the sclera can be assessed by comparing the scleral and choroidal levels. There were no significant differences between the scleral and choroidal levels for PEDF and TGF β 1 and only a modest difference for sFlt-1. This suggests that with constant protein production in the episclera, the sclera does not provide a barrier to entry of PEDF or TGF β 1 into the choroid and is only a modest barrier to sFlt-1, possibly due to its larger size. In contrast, there were major differences between choroidal and retinal levels for each of the three proteins (Fig. 3), indicating a substantial barrier to entry into the retina from the periocular space for each of these proteins.

FIG. 3. Levels of protein in ocular tissues after a periocular injection of corresponding vector. Forty-eight (48) h after periocular injection of 5 μ L containing 5×10^9 viral particles of AdPEDF.11 or AdTGF β 1.10, levels of PEDF or TGF β 1 were measured in ocular tissues by enzyme-linked immunosorbent assay. An excerpt of the data from Figure 2 is provided in 3A to facilitate comparison of sFlt-1 with PEDF and TGF β 1. The bars represent the mean (\pm standard error of the mean) calculated from ≥ 6 experimental values. For each tissue, the protein level for each of the three proteins was significantly greater after the periocular injection of the vector, compared to the uninjected control. Statistical comparisons using analysis of variance with Dunnett's correction for multiple comparisons showed no significant difference between scleral and choroidal levels for PEDF, TGF β 1, and sFlt-1, but there was a significant difference between choroidal and retinal levels for each of the three proteins ($P < 0.005$).

Periocular injection of PEDF protein

Measurement of PEDF levels (pg/ μ g total protein) in the sclera, choroid, and retina were done at several time points after a periocular injection of 5 μ L containing 3.65 μ g of human recombinant PEDF. Peak levels of 55.88 and 28.60 occurred in the sclera and retina, respectively, at 2 h after the injection (Fig. 4). At 6 h after the injection, PEDF levels were 21.47 and 10.72 in the sclera and choroid, respectively, and by 24 h, levels were 0.45 and 0.10. Levels in the retina were very low at all time points.



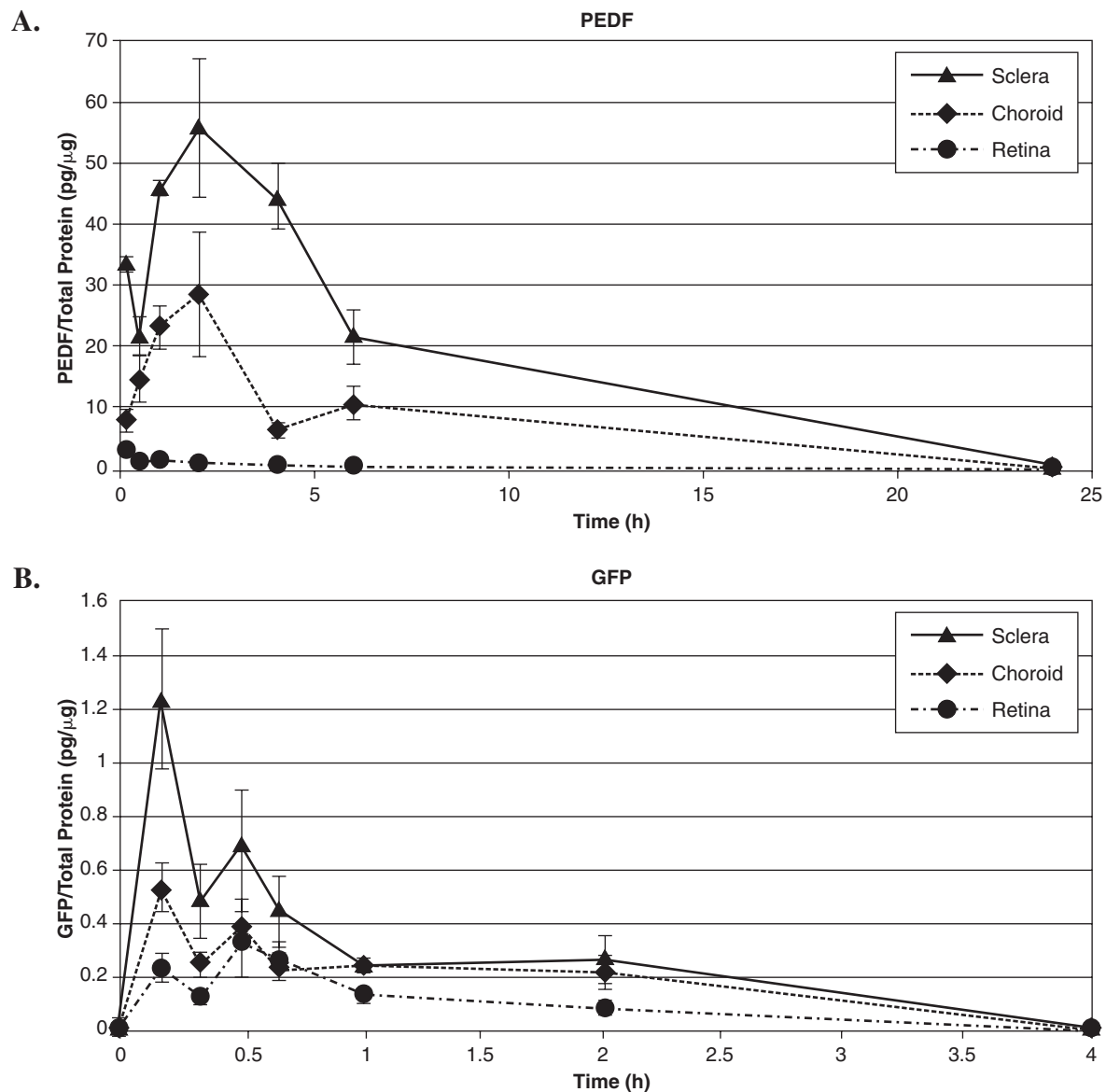


FIG. 4. Trans-scleral delivery of pigment epithelium-derived factor (PEDF) or green fluorescent protein (GFP). Adult C57BL/6 mice ($n = 10$ for each dose) were given a $5\text{-}\mu\text{L}$ periocular injection containing $3.65\ \mu\text{g}$ of human recombinant PEDF or $3.65\ \mu\text{g}$ of recombinant GFP in 1 eye and no injection in the fellow eye. Mice were euthanized and ocular tissue samples were dissected at 10 or 20 min, or 1, 2, 5, or 24 h after a PEDF injection (**A**) or 10, 30 min, or 1, 2, or 4 h after GFP injection (**B**). The total amount of protein in each sample was measured and GFP or PEDF were measured by enzyme-linked immunosorbent assay. (**A**) PEDF levels peaked in sclera and choroid at 2 h after injection were still significantly above baseline at 6 h and returned to baseline between 6 and 24 h. Levels in choroid were quite substantial, with a peak of about $30\ \text{pg}/\mu\text{g}$ protein at 2 h after injection and levels above or near $10\ \text{pg}/\mu\text{g}$ for about 6 h. Levels in the retina were significantly above baseline at $1\ \text{pg}/\mu\text{g}$ for up to 2 h. (**B**) GFP levels peaked very early at 10 min (the first measurement) in sclera and RPE/choroid and returned to baseline by 4 h. In contrast to PEDF, the levels of GFP in the retina were significantly above baseline at early time points and peaked 30 min after injection. A computational model of GFP transport in the posterior segment was used to determine values for the transport parameters.²⁰ The simulation results fit well with the experimental data.

Periocular injection of GFP

GFP is completely foreign to mice and there are unlikely to be any specific interactions with endogenous proteins that would alter its behavior in mouse tissues. Ten (10) min after a periocular

injection of $5\ \mu\text{L}$ containing $3.65\ \mu\text{g}$ of GFP, it was detectable in the sclera and choroid, but not in the retina. Levels in the retina peaked at 30 min after injection, and at 4 h after injection, GFP was not detectable in any intraocular tissue.

DISCUSSION

Periocular injections are a safe way to provide local delivery of drugs and proteins to the eye, but very little is known about the pharmacokinetics of penetration into the eye after periocular injection. In previous studies, our laboratory demonstrated that proteins as large as 75 kDa are able to penetrate the sclera and suppress choroidal neovascularization after the transduction of periocular tissue with Ad vectors.^{10,11} In this study, we used the same vectors that were used in those previous studies to measure the levels of sFlt-1 and PEDF that were achieved after a periocular injection of AdsFlt-1.10 or AdPEDF.11. We found that the scleral production of PEDF was quite high after a periocular injection of AdPEDF.11, while scleral production of sFlt-1 was substantially lower after a periocular injection of AdsFlt-1.10. Compared to the situation with AdPEDF.11, a periocular injection of AdTGF β .10 also resulted in relatively low levels of TGF- β in the sclera. Despite these differences in starting levels, scleral penetration was excellent for PEDF and TGF- β and was less, but still good, for sFlt-1, possibly due to the larger size of sFlt-1 relative to PEDF and TGF- β . For all three proteins, there was a substantial difference between the choroidal and retinal levels, indicating that a barrier exists for the entry of these three proteins into the retina from the periocular space.

The measurements obtained after periocular gene transfer allow for the assessment of steady-state levels of protein achieved in intraocular tissue compartments with sustained production of proteins in the sclera. Periocular injection of proteins provides a means for the dynamic assessment of protein penetration. Peak levels were achieved in the sclera and choroid 2 h after a periocular injection of human recombinant PEDF. Unlike the situation with continuous production of PEDF in the sclera, there was a significant difference between scleral and choroidal levels after an intraocular injection, indicating that while scleral penetration is excellent, it is not instantaneous. There were still good levels of PEDF in the choroid 6 h after injection that returned to baseline between 6 and 24 h. Compared to the periocular injection of PEDF, the time course was compressed after the periocular injection of GFP, with peak levels occurring in the sclera and choroid at 10 min after injection and returned to baseline by 4 h. Peak levels of GFP occurred in

the retina 30 min after injection and were 64.3% of peak choroidal levels. Peak retinal levels after the injection of PEDF were 8.6% of peak choroidal levels, indicating that the barrier to entry into the retina from the choroid was substantially less for GFP, compared to PEDF. GFP is a completely foreign protein in the mouse and specific binding sites for GFP are unlikely to be present, whereas binding sites for PEDF are present.¹⁷⁻¹⁹ This may explain why PEDF remains in the choroid longer than GFP. It is possible that lack of binding also contributes to GFP entry into the retina from the choroid, although its smaller size (27 vs. 50 kDa) is also likely to be important.

A computational model of the transport of GFP through the layers of the posterior segment following a periocular injection was developed to identify the barriers to transport.²⁰ The model characterizes each layer (i.e., sclera, choroid, and retina) separately, with each layer having a different GFP diffusivity, clearance rate, and interstitial fluid volume. The resistances to transport between each layer due to the presence of membranes are also included. The model results matched the GFP experimental results well (Fig. 4B), and values for several of the transport parameters (e.g., membrane resistances to GFP, clearance rates) were quantified. The internal limiting membrane and the diffusive barrier of the retina itself (which is thicker than the sclera and choroid) were found to form the bulk of the resistance to transport of GFP within the layers of the posterior segment. For a larger protein, such as PEDF or sFlt-1, the model predicts a modest rise in the diffusive barriers of the sclera and choroid, but a significant increase in the barrier resistances between the layers, especially the RPE.

The mouse eye is very small relative to the human eye, and in order to apply trans-scleral penetration results obtained in mouse eyes to human eyes, it is useful to know how they compare with regard to relevant structures. The human sclera is thinnest beneath the attachments of the rectus muscles (300 μm) and at the equator (600 μm) and thickest around the optic nerve (800 μm).²¹ This is roughly a thirtyfold difference from the measurements obtained in mouse eyes. The human choroid is thickest beneath the fovea (300 μm) and thinnest at the ora serrata (100–150 μm), roughly, a fifteenfold difference from the mouse. While these differences are substantial and must be taken into consideration when determining

how the results apply to humans, it should be noted that the sclera and choroid are very low-density tissues with high water content. Thickness is unlikely to be the major factor influencing the passage of material through these highly porous tissues. Choroidal blood flow and biochemical characteristics of the tissues, things that are likely to be more similar between humans and mice than thickness, are likely to be more important determinants of penetration. In support of this, periocular injections of AdPEDF had similar effects on choroidal neovascularization in pigs and mice.^{10,12}

Thus, while caution is always advised when applying results in animal models to human disease, there are potential clinical implications for the findings in this study. Proteins as large as 50–75 kDa can be delivered to the choroid by a periocular injection or periocular gene transfer in mice and pigs. This suggests that choroidal neovascularization can be treated by the periocular delivery of agents, and efficacy studies support this contention.²² However, there may be differences between the sclera and choroid of young mice and pigs and those of elderly humans. Unless those difference have a major effect on penetration, one would predict that small proteins and other small molecules could be delivered by periocular delivery, but pharmacokinetic studies in humans will be needed to determine if this is definitely the case. In contrast, penetration of PEDF, sFlt-1, and TGF- β 1 into the retina was poor, even with sustained expression in episclera.

CONCLUSIONS

The feasibility of treating retinal diseases by the periocular delivery of agents is less certain than the treatment of choroidal diseases; however, this is likely to depend upon the size and potency of the therapeutic agents. Compared to PEDF, the relative levels of GFP entering the retina from the choroid were substantially greater and, therefore, additional work is needed to identify characteristics that may promote the entry of drugs and proteins into the retina from the periocular space.

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