Influence of Molecular Weight on Intracameral Dextran Movement to the Posterior Segment of the Mouse Eye

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PURPOSE. Uveoscleral outflow provides a potential pathway to the posterior segment for drug delivery. In this study, the influence of molecular weight on the intraocular distribution of dextran molecules after intracameral injection in the mouse eye was investigated.

METHODS. The anterior chambers of the eyes of 64 anesthetized NIH Swiss mice were perfused with various fluorescent dextran solutions (10, 40, 70, and 500 kDa) at 500 nL/min for 10 minutes. At 10, 20, or 60 minutes after the initiation of the anterior chamber perfusion, the mice were killed and tissue obtained for evaluation by fluorescence microscopy.

RESULTS. Each of the different molecular weight dextrans were visible in the anterior chamber of the mouse eye after the termination of the experiments. The 10-kDa dextran was observed in the supraciliary space and the supraciliary sclera after 10 minutes and in the anterior sclera after 60 minutes of perfusion. The 40-kDa dextran was detected in the supraciliary space and the anterior sclera after 10 minutes and in the anterior choroid and sclera after 20 and 60 minutes, but not in the posterior segment. The 70-kDa dextran was observed in the supraciliary space and anterior choroid after 10 minutes. After 20 minutes, it was visible in the equatorial choroid. After 60 minutes, it was observed in the posterior choroid. The 500-kDa dextran was observed in the supraciliary space and the anterior choroid after 10 minutes and in the supraciliary sclera at 20 minutes. At 60 minutes, 500-kDa dextran was observed in the equatorial choroid, but not farther toward the posterior.

CONCLUSIONS. The influence of molecular weight on the redistribution of macromolecules from the anterior chamber to the posterior globe in the mouse eye appears to be similar to primate eyes. These similarities include passage of all size dextrans through the proximal uveoscleral pathway, the dependence of the extent of posterior movement on the size of the dextran, and the absence of large dextran entry into the distal uveoscleral pathway. (Invest Ophthalmol Vis Sci. 2004; 45:480–484) DOI:10.1167/iovs.03-0462

Aqueous humor drains from the anterior chamber predominantly through two routes: the trabecular meshwork outflow pathway and the uveoscleral outflow pathway. Aqueous humor exiting by the uveoscleral route traverses through the interstitial spaces of the iris root and the ciliary muscle, enters the suprachoroidal space and anterior choroid, and finally passes through the sclera. Evaluation of tracer distribution 30 minutes after intracameral introduction of various-sized dextrans into cynomolgus monkey eyes found that a greater proportion of 4-kDa dextran was recovered from the anterior sclera than either 40-kDa dextran or 150-kDa dextran. In contrast, a greater proportion of 40-kDa dextran was recovered from the anterior uvea than either 4-kDa dextran or 0.4-kDa dextran. However, the physiologic and biological basis of this differential transport within the uveoscleral outflow route remains poorly understood.

Recent studies have demonstrated several similarities between the primate eyes and the mouse eye that raise the possibility that the mouse may be suitable for investigation of differential molecular transport within the uveoscleral outflow pathway. These include a radially symmetric iris, an endothelial-cell–lined Schlemm’s canal, a well-differentiated trabecular meshwork, a distinct ciliary muscle, well-developed ciliary processes, and a morphologically similar uveoscleral outflow pathway. Furthermore, intraocular pressure (IOP) in the mouse eye is similar to that in the human eye, and there is evidence of both trabecular and uveoscleral outflow. Although some differences between mouse and primate uveoscleral outflow are likely, the recent maturation of transgenic mouse technology provides an opportunity for direct study of the biological mechanism(s) that regulate movements in the uveoscleral pathway. However, the utility of the mouse eye as a model in which to study uveoscleral outflow remains limited, because the relationship between molecular size and the posterior uveoscleral movement of macromolecules in the mouse eye is not known. Thus, the present study was undertaken to investigate this relationship by observing the progression of various-sized dextran molecules as they moved from the anterior chamber posteriorly through the uveoscleral outflow pathway of the mouse eye.

METHODS

Rationale

Mouse eyes were perfused for 10 minutes with buffered saline containing one of four different molecular mass dextrans with covalently attached fluorochrome molecules and lysine residues. At 10, 20, or 60 minutes after the initiation of anterior chamber perfusion, the mice were quickly killed and transcardially perfused with an aldehyde fixative that cross-linked the intraocular tracer molecules to tissue proteins. Thus, the tracer molecules rapidly became immobilized. Sections of the eyes were then evaluated by fluorescence microscopy.

Tracer Preparation

Four different molecular mass lysine-fixable dextrans conjugated to fluorescent dyes were obtained from Molecular Probes, Inc. (Eugene,
OR: 10 kDa Oregon Green, 40 kDa fluorescein, 70 kDa Texas red, and 500 kDa fluorescein. In each case, a range of molecular masses was represented as follows: 10 kDa included 7 to 12 kDa, 40 kDa included 35 to 50 kDa, 70 kDa included 60 to 90 kDa, and 500 kDa included 350 to 650 kDa.4,13 The dextrans were dissolved in 0.1 M phosphate-buffered saline (PBS) to stock solutions at a concentration of 2.5 mg/mL and stored at −20°C. Dextrins with different fluorochromes were used, because dextrans with the same fluorochrome for all molecular sizes used in this study were not available. Initial studies showed that the intensity of fluorescence from the Oregon Green—Texas red labeled dextrans were similar and had markedly brighter fluorescence than the fluorescein-labeled dextrans. Hence, to obtain equivalent fluorescent signals, the appearance in the uveoscleral pathway of increasing concentrations of 70 kDa dextran conjugated to fluorescein was compared to the appearance of 70 kDa dextran conjugated to Texas red. It was determined that perfusion with 750 mg/mL 70 kDa fluorescein dextran produced a distribution of fluorescence that was most similar in appearance to that after perfusion with 10 mg/mL Texas red 70 kDa dextran. Hence, the perfusion solutions for each experiment were prepared with a final concentration of 10 μg/mL (10 kDa Oregon Green dextran, 70 kDa Texas red dextran) or 750 μg/mL (40- and 500-kDa fluorescein dextran) in PBS. Immediately prior to anterior chamber perfusion, the solutions were passed through a filter with a pore size of 0.2 μm (GD/X; Whatman, Marlborough, MA) to avoid injection of aggregated dextran molecules.

**Animals and Anesthesia**

Animals used in this study were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Sixty-four NIH white Swiss mice were anesthetized by intraperitoneal injection of a mixture of ketamine (100 mg/kg, Ketaset; Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (9 mg/kg, Tranquilo-Ved; Vedco, Inc., St. Joseph, MO). Because the duration of anesthesia was approximately 45 minutes, a booster dose of anesthesia was injected approximately 30 minutes after the initial dosage during the experiments that lasted 60 minutes. After confirmation of loss of response to tail pinch, the mice were positioned on an observation platform and held in position using wire loop head, nose, and tail holders. Both eyes received 1 drop of PBS to avoid corneal desiccation.

**Perfusion of the Dextran Tracers**

Each dextran solution was perfused into the anterior chamber of a mouse eye through a glass microneedle. The microneedle was prepared from a borosilicate glass tubing using a pipette puller (P-87; Sutter Instruments, Novato, CA) and a tip bevel of 30° was produced using a microgrinder (Micropette Beveler; World Precision Instruments [WPI], Inc., Sarasota, FL). The microneedle was connected to a pressure transducer that was connected to a microsyringe. The microsyringe was fitted into a microprocessor-controlled motorized syringe drive (UltraMicroPump II; WPI, Inc.). The system was filled with the tracer solution. Care was taken to prevent air bubbles.

The experiment was performed while the anterior segment was viewed through a microscope. In addition to protecting against desiccation, the drop of PBS placed on the eye enhanced the view of the anterior chamber. The tip of the needle was placed in the drop of PBS over the left eye and then inserted into the anterior chamber by means of a micromanipulator. Contact with the iris tissue was avoided. IOP was measured as previously described.14

To maintain constant IOP during the dextran infusion, a second microneedle was then inserted into the anterior chamber. The microneedle was connected to an open reservoir by saline-filled tubing to allow an adequate outflow of the anterior chamber volume. This arrangement allowed IOP to be maintained within the range of normal for this mouse strain (13–18 mm Hg).

Initially, the aqueous humor was replaced by rinsing the anterior chamber with 5.0 μL of the tracer solution over the course of 10 seconds. During the rinse, the pressure was maintained less than 20 mm Hg by lowering the reservoir. This rinsing procedure was repeated two more times with a 5- to 10-second rest between rinses. As the total volume of the anterior chamber in this mouse strain is 5 to 6 μL,15 each rinse was equivalent to the volume of the anterior chamber. Continuous perfusion of the anterior chamber was then performed with a constant speed of 500 nL/minute for a period of 10 minutes. After tracer perfusion, the microneedles were maintained in the anterior chamber for a total of 10, 20, or 60 minutes from the initiation of perfusion. Three or four eyes were analyzed for each experimental condition, and 4 to 15 sections were examined for each condition.

**Histologic Analysis**

After perfusion, each mouse was killed by CO2 inhalation. One drop of fixative containing 2% formaldehyde and 0.5% glutaraldehyde was then instilled on the perfused eye. Next, the mouse was transcardially perfused through the left ventricle with 0.1 M PBS followed by fixative containing 2% formaldehyde and 0.5% glutaraldehyde in PBS (pH 7.4). After enucleation, the eyes were postfixed in the same fixative solution for 1 hour and then cryoprotected by passage through 10%, 20%, and 30% sucrose in PBS. After placing the tissue into molds containing tissue-freezing medium (TFM; Triangle Biomedical Sciences, Durham, NC), the tissue was snap frozen by placing the mold into a slurry of acetone and dry ice. Cryostat sections (10 μm thick) were cut, and the sections were placed on gelatin-coated glass slides. The slides were analyzed by fluorescence microscopy (Eclipse E800; Nikon Inc., Melville, NY), and photographs were taken with a cooled digital camera (SPOT Digital Camera System; Diagnostic Instruments, Inc., Sterling Heights, MI). To identify the tracer within the anatomic structures and to differentiate tracer signal from the autofluorescence of the tissue, the sections of the perfused eye were compared with sections obtained from the nonperfused control eye.

**RESULTS**

In all perfused eyes, fluorescence was observed in the anterior chamber mostly at the anterior iris surface, but also in smaller amounts in the corneal endothelium, in the chamber angle, and at the posterior surface of the iris. The results are summarized in Table 1.

**10-kDa Dextran**

After 10 and after 20 minutes, fluorescence was present in the suprachoroidal space and in the suprachoroidal sclera (adjacent to the ciliary body; Figs. 1A, 1B). Fluorescence observed in the sclera was punctate and weaker in intensity than the fluorescence in the suprachoroidal space. At 60 minutes after the start of perfusion, punctate fluorescence was present in the anterior sclera, immediately behind the ora serrata (Fig. 1C).

**40-kDa Dextran**

Ten minutes after initiation of the perfusion, fluorescence was visible in the suprachoroidal space, and the suprachoroidal and anterior sclera (Fig. 2A). After 20 and 60 minutes, the fluorescent signal extended to the anterior choroid and was visible in the adjacent sclera (Figs. 2B, 2C).

**70-kDa Dextran**

The 70-kDa dextran was observed in the anterior chamber, the suprachoroidal space, and the anterior choroid immediately after 10 minutes of anterior chamber perfusion (Fig. 3A). After 20 minutes, intense fluorescence was present in the choroid, extending to the equator region (Fig. 3B). Sixty minutes after initiation of the anterior chamber perfusion, the fluorescence was present in all segments of the mouse eye extending from...
the supraciliary space to the choroid, including the posterior
lobe (Figs. 3C, 3D). No fluorescence was observed in the
retina, the sclera, or the optic nerve tissue.

500-kDa Dextran

The 500-kDa dextran was observed in the anterior
chamber, the supraciliary space, and, at lower intensity, in the anterior
choroid 10 minutes after initiation of the anterior chamber
perfusion (Fig. 4A). After 20 minutes, fluorescence was present
in the supraciliary sclera (Fig. 4B). At 60 minutes, the dextran
was detected in the anterior choroid and in the adjacent sclera
(Fig. 4C). Fluorescence extended to the equatorial choroid, but
not farther toward the posterior.

**DISCUSSION**

The results of this study show that redistribution of intracam-
eral dextran within the mouse uveoscleral outflow pathway
depends on the molecular mass of the dextran tracer and the
time interval after its placement within the anterior chamber.
The 10-, 40-, and 500-kDa dextrans readily passed into the
supraciliary space, but were not observed posterior to the
equator. In contrast, the 70-kDa dextran entered the supraca-
iliary space, then the choroid, and then the posterior choroid.
Although 500-kDa dextran entered the proximal uveoscleral
outflow pathway, it was not observed farther toward the pos-
terior than the equatorial choroid. Moreover, unlike the smaller
dextrans, 500-kDa dextran appeared to have accumulated at
the anterior choroid and sclera. These results indicate that the
efficiency of dextran movement from the anterior chamber to
the posterior eye increases with molecular mass up to a certain
point and then decreases with a further increase in mass.

Several aspects of the experimental design support the
validity of these observations. First, previous studies in the
mouse eye indicate that the distribution of dextran is unlikely
to be influenced by nonspecific binding to tissue elements, as
it is readily washed out by normal aqueous humor flow.5,9

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**FIGURE 1.** Distribution of 10-kDa dextran conjugated to Oregon
Green (A) 10, (B) 20, or (C) 60 minutes after initiation of the perfusion.
(D) Section from a control eye that did not receive dextran perfusion.
These images illustrate the results in four eyes (6 sections total) at 10
minutes, four eyes (4 sections) at 20 minutes, and three eyes (15
sections) at 60 minutes. R, retina; S, sclera; CB, ciliary body. Magnifi-
cation, ×100.

**FIGURE 2.** Distribution of 40-kDa dextran conjugated to fluorescein
(A) 10, (B) 20, or (C) 60 minutes after initiation of the perfusion. (D)
Section from a control eye that did not receive dextran perfusion.
These images illustrate the results in three eyes (eight sections total) at
10 minutes, three eyes (nine sections) at 20 minutes, and four eyes
(eight sections) at 60 minutes. R, retina; S, sclera; CB, ciliary body. Magnification, ×100.
dextran perfused within the anterior chamber of the mouse appears to have ready access to the uveoscleral outflow pathway and also can be observed in the posterior portion of this pathway.

In contrast to the 70-kDa dextran, the 10- or 40-kDa dextrans entered the suprachoroidal space and the anterior sclera, but did not move farther toward the posterior. These observations suggest that the smaller tracers readily entered the proximal uveoscleral outflow pathway and then largely exited it, most likely through the sclera, before accumulating in the posterior. In contrast, 70-kDa dextran was not observed in the suprachoroidal sclera at any of the time points examined and traversed through the uveoscleral outflow pathway to the posterior pole. Thus, the smaller dextrans readily passed from the anterior uveal tissue into the anterior sclera, whereas the 70-kDa dextran did not. This effect most likely reflects the influence of the dextran’s molecular mass, as 40-kDa dextran was present in the anterior sclera at 10 and 20 minutes, whereas the 10-kDa dextran was not observed in the anterior sclera until 60 minutes after the start of perfusion. This result is similar to the observation of more efficient transfer of intracamerally perfused 4-kDa dextran to anterior sclera in the monkey eye than either 40- or 150-kDa dextran.3

The 10 kDa dextran disappeared quickly from the suprachoroidal space, possibly by diffusion, reabsorption, or both. Diffusion through scleral tissue may occur immediately after perfusion. Pooling in the looser connective layers may cause the punctate fluorescent pattern in the center of the sclera. Further, the small molecules may be reabsorbed by uveal vessels or pass through the perivascular or perineural spaces within the sclera.16,19 However, a study comparing the intraocular transport of 4-kDa dextran with larger dextrans concluded that absorption of this dextran was minimal.5 Further studies of the diffusion of small molecules in the anterior uveoscleral outflow pathway are needed.

In the mouse eye, the 500-kDa dextran was present in the anterior choroid, and, after 60 minutes, within the equatorial choroid and anterior choroid. There was no fluorescence present posterior to the equator. No tracer was observed pos-

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**FIGURE 3.** Distribution of 70-kDa dextran conjugated to Texas red in the anterior segment (A) 10, (B) 20, or (C) 60 minutes after initiation of the perfusion. Fluorescence also was present in the posterior choroid at 60 minutes after initiation of the perfusion (D). This appeared as a thin bright line between the sclera and retina near the optic nerve head. These images illustrate the results in three eyes (6 sections total) at 10 minutes, four eyes (5 sections) at 20 minutes, and three eyes (10 sections) at 60 minutes. R, retina; S, sclera; CB, ciliary body; M, muscle; ON, optic nerve. Magnification, ×100.

Second, experiments have shown that dextrans conjugated to fluorescent dyes are stable within such biological environments as tissues and fluids.15 Third, previous studies examining the movement of 4- to 150-kDa dextrans in monkey eyes found that the extent of movement varies with molecular mass.15 Finally, the tissue distribution of the lysine-conjugated dextrans was stabilized by aldehyde-mediated cross-linking of these tracers to protein elements within the tissue during fixation. Thus, because it is unlikely that the cross-linked tracer moved during tissue processing, the distribution revealed by fluorescence microscopy is likely to reflect distribution in vivo. These considerations support the present results as accurately representing the tissue distribution of the perfused dextrans.

The present study found that 70-kDa dextran Texas red perfused through the anterior chamber readily entered the mouse uveoscleral outflow pathway and moved toward the posterior within 60 minutes. These results are essentially the same as those in a recent study in which the uveoscleral outflow pathway was delineated in the mouse eye after slow intracameral injection of 70-kDa dextran-tetramethylrhodamine.9 In the earlier study, intense fluorescence was observed to extend to the anterior choroid after 20 minutes and posteriorly to the equator by 60 minutes. Because dextran conjugated to Texas red has a neutral charge whereas dextran conjugated to tetramethyl rhodamine has a negative charge, this similarity of results with these two tracers suggests that the charge difference did not significantly affect the efficiency of macromolecular movement within the uveoscleral pathway. This is consistent with the previous observation that transscleral permeability is influenced more by molecular radius than by molecular mass or charge.16 The present results also are similar to those in which albumin, a negatively charged glycoprotein with a molecular mass of 66 kDa, was used to examine the uveoscleral outflow in monkeys.1,17 In that study, it was observed that after perfusion of the anterior chamber for 30 minutes, the dissected ocular tissues contained significant amounts of tracer in the anterior sclera, the iris, and in the ciliary body, but not in the posterior sclera.17 Thus, 70-kDa dextran perfused within the anterior chamber of the mouse appears to have ready access to the uveoscleral outflow pathway and also can be observed in the posterior portion of this pathway.

In contrast to the 70-kDa dextran, the 10- or 40-kDa dextrans entered the suprachoroidal space and the anterior sclera, but did not move farther toward the posterior. These observations suggest that the smaller tracers readily entered the proximal uveoscleral outflow pathway and then largely exited it, most likely through the sclera, before accumulating in the posterior. In contrast, 70-kDa dextran was not observed in the suprachoroidal sclera at any of the time points examined and traversed through the uveoscleral outflow pathway to the posterior pole. Thus, the smaller dextrans readily passed from the anterior uveal tissue into the anterior sclera, whereas the 70-kDa dextran did not. This effect most likely reflects the influence of the dextran’s molecular mass, as 40-kDa dextran was present in the anterior sclera at 10 and 20 minutes, whereas the 10-kDa dextran was not observed in the anterior sclera until 60 minutes after the start of perfusion. This result is similar to the observation of more efficient transfer of intracamerally perfused 4-kDa dextran to anterior sclera in the monkey eye than either 40- or 150-kDa dextran.3

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In the mouse eye, the 500-kDa dextran was present in the anterior choroid, and, after 60 minutes, within the equatorial choroid and anterior choroid. There was no fluorescence present posterior to the equator. No tracer was observed pos-

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**FIGURE 4.** Distribution of 500-kDa dextran conjugated to fluorescein (A) 10, (B) 20, or (C) 60 minutes after initiation of the perfusion. (D) Section from a control eye that did not receive dextran perfusion. (D) Distribution of 500-kDa dextran conjugated to fluorescein (A) 10, (B) 20, or (C) 60 minutes after initiation of the perfusion. (D) Section from a control eye that did not receive dextran perfusion. (D) Section from a control eye that did not receive dextran perfusion.
terior to the ciliary body. Moreover, the distribution of fluorescence in the sections from eyes maintained for 20 or 60 minutes after perfusion was essentially the same, which indicates that 500-kDa dextran accumulated in the anterior uveoscleral pathway and suggests that there may be an upper size limit for the access of macromolecules to the posterior choroid. Several observations suggest that an upper size limit exists in the monkey eye, as well. After intracameral perfusion of the same amounts of various-sized dextrans, 150-kDa dextran molecules were present in the posterior sclera at a lower concentration than 40- and 4-kDa dextrans. Similar results were obtained after anterior chamber perfusion of monkey eyes with a gelatin solution containing colloidal thorium dioxide particles (~0.01 μm diameter) and latex spheres that were 0.1, 0.5, and 1.0 μm in diameter. Three hours later, both Thorotrast particles and 0.1-μm spheres were observed in the suprachoroid of the macular region, whereas the larger spheres were excluded. As the tracer types and the experimental designs of these studies differed from those in the present study, it is not possible to say whether the upper size limits of the uveoscleral outflow systems of the mouse and monkey eyes are different. Further studies comparing uveoscleral outflow in mouse and monkey eyes with the same tracers and experimental design will resolve this issue.

A possible limitation of the present results is that higher concentrations were used for the 40- and 500-kDa dextrans than for the 10- and 70-kDa dextrans. This difference served to provide comparable fluorescence signal intensities within the anterior segment tissues, as the fluorescence intensity of fluorescein was less than that of Oregon Green or Texas red. Because all the tracers used in this study were hydrophilic, it is not likely that tracer precipitation was a problem. It is possible, however, that the hydrodynamic properties of the solutions were influenced by the different dextran concentrations. Further studies of the effects of concentration would help to clarify this point. Other limitations of the present study include that only various-sized dextrans were examined and that the analysis was essentially qualitative. Previous studies have shown that the movement of linear dextran molecules across the sclera differs from similar-sized globular proteins. Hence, further comparative studies using quantitative measurements are needed to determine whether dextran movement in the mouse uveoscleral pathway differs from particulate tracers, microspheres, or globular proteins. Finally, because tracers within the monkey uveoscleral outflow pathway accumulate within the retrobulbar orbital tissues, similar evaluation of tracer accumulation within mouse eye retrobulbar tissue may provide further insight into the function of the mouse uveoscleral outflow pathway.

In conclusion, the influence of molecular mass on the redistribution of macromolecules from the anterior chamber to the posterior globe in the mouse eye appears to be similar to primate eyes. These similarities include passage of all size dextrans through the proximal uveoscleral pathway, the dependence of the extent of posterior movement on the size of the dextran, and the absence of large dextran entry into the distal uveoscleral pathway. Hence, the mouse model may be useful in the study of the biological basis of uveoscleral outflow regulation.

References