The Effect of Intraocular Pressure on Human and Rabbit Scleral Permeability

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Purpose. The purpose of this study was to evaluate the effects of intraocular pressure on the permeability of human and rabbit sclera to water, dexamethasone, and carboxyfluorescein.

Methods. Scleral sections excised from moist-chamber-stored human globes or eyes obtained from euthanatized New Zealand White rabbits were mounted in a perfusion chamber that can create a transscleral pressure that simulates an intraocular pressure. A small depot of drug (100 μl) was added to the episcleral surface while perfusing an irrigating solution slowly across the choroidal side. The perfusate was collected and scleral permeability calculated. Experiments were performed at 0, 15, 30, and 60 mm Hg for each compound in human and rabbit tissue.

Results. Analysis of variance showed a significant effect of intraocular pressure on both human and rabbit scleral permeability. Human scleral permeability was decreased by as much as a factor of two for water ($P = 0.0004$), dexamethasone ($P < 0.0001$), and carboxyfluorescein ($P = 0.0064$) at elevated intraocular pressures. Rabbit scleral permeability was similarly affected by elevated intraocular pressure for water ($P = 0.0039$), dexamethasone ($P = 0.0001$), and carboxyfluorescein ($P = 0.0016$).

Conclusions. This study shows that simulated intraocular pressure ranging from 15 to 60 mm Hg can decrease scleral permeability to small molecules by one half when compared with the sclera with no pressure applied. (*Invest Ophthalmol Vis Sci.* 1999;40:3054-3058)

Retinal disease is one of the major causes of blindness. The treatment of retinal disease is to some degree limited by the difficulty of delivering drugs to target tissues in the posterior eye. Traditional routes of local ophthalmic delivery (i.e., topical) do not yield therapeutic drug levels in the posterior tissues of the eye. Although systemic administration can deliver drugs to the posterior eye, the large systemic doses necessary are often associated with side effects. The sclera offers another vector to obtain therapeutic vitreous and retinal drug concentrations by either subconjunctival or retrobulbar injection.[1-3] Delivering drugs across the permeable sclera would be safer and less invasive than intravitreal devices yet could provide a more effective retinal dose than systemic or topical delivery.

Although past in vitro studies have reported scleral permeability for compounds with a wide range of molecular weights, the experiments were performed using chambers that do not impose a transscleral pressure to simulate the intraocular pressure observed in an intact eye.[4,5] For this study, we designed a chamber that emulates depot delivery from the scleral surface. The chamber design allows the experimenter to impose a transscleral pressure through a water column (Fig. 1). The simulated intraocular pressure can be controlled by varying the height of the water column, measured by an attached pressure transducer. The *choroidal* hemichamber—representing the choroidal tissues—is perfused at a slow rate, whereas the *episcleral* hemichamber is held static, similar to a drug added to Tenon's space and directly exposed to the sclera.

The purpose of this study was to determine the effect of intraocular pressure on scleral permeability of low-molecular-weight compounds, including water, after a depot application to the episclera. Scleral permeability constants (K_{trans}) for water, dexamethasone, and carboxyfluorescein were measured at simulated intraocular pressures of 0, 15, 30, and 60 mm Hg.

Methods

Scleral tissue was obtained from 66 human donor eyes (Georgia Eye Bank, Atlanta) that had been stored in moist chambers for an average ± SD of 4.5 ± 1.9 days (mean age, 55.0 ± 15.8 years). For rabbit studies, sclera was obtained from 55 eyes of New Zealand White rabbits weighing 5.0 to 5.5 kg, which were anesthetized and then killed by intracardiac injection of sodium pentobarbital (97.2 mg/kg). All animal protocols for these experiments conformed to the Guiding Principles in the Care and Use of Animals (Department of Health, Education and Welfare Publication, NIH 80-23) and the ARVO Resolution for the Use of Animals in Ophthalmic and Vision Research. The eye was enucleated, and the extraocular tissues, including conjunctiva and extraocular muscles, were carefully removed. The episclera and uvea were removed with a cotton swab to isolate the bare sclera. Scleral disks of 15 to 20 mm in diameter were excised from the superior temporal section of globe, 12 to 15 mm posterior to the limbus.[3]

The excised sclera was mounted choroid side down in a specially designed Lucite perfusion chamber, in which the sclera was mounted horizontally (Fig. 1). The sclera was clamped between two 2.5-mm-wide (and approximately 1-mm-thick) cylindrical rings (Sylgard; Dow Corning, Midland, MI) cut to the size of the chamber opening to prevent lateral leakage and scleral edge damage. Chambers with a 7-mm aperture were used for rabbit experiments, whereas at times a 10-mm aperture was used for human experiments and at others a 7-mm aperture was used. BSS Plus (Alcon Laboratories, Fort Worth, TX) was perfused through the lower hemichamber (500 μl volume) at a rate of 0.03 ml/min. Fluid mixing was

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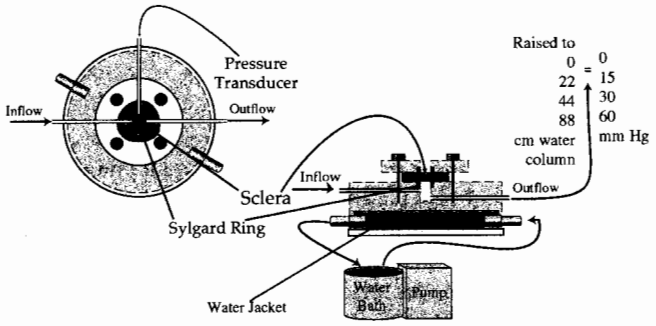

FIGURE 1. The perfusion chamber used for measuring scleral permeability under simulated intraocular pressure. Sclera was mounted in a horizontal perfusion setup (episcleral side up) to allow for an intraocular pressure to be simulated by creating a positive water column with the outflow tube. The compound to be tested was added to the upper, episcleral hemichamber. The lower, choroidal hemichamber has a continual flow and is mixed by a magnetic stir bar with the perfusion chamber placed on a magnetic stir plate.

achieved in the lower hemichamber with a magnetic microstir bar, with the chamber resting on a magnetic stir plate. The tissue was perfused for 15 to 30 minutes to verify that no leaks were present before applying a test compound to the surface. At times, particularly with rabbit experiments, the cylindrical rings would not form a complete seal around the circumference of the exposed region, causing fluid to leak laterally along the surface of the sclera. Thus, carboxyfluorescein (or Evan's Blue when measuring carboxyfluorescein permeability) was added to the upper hemichamber to help visualize leakage. Experiments in which leakage occurred were not included in the results.

The test compound, adjusted to a total volume of 100 μl with BSS Plus, was added to the episcleral surface 15 to 30 minutes after the sclera was mounted in the chamber. The upper hemichamber containing the test compound was covered with parafilm and sealed with silicone grease (Dow Corning) along the edges of the exposed area of the chamber to prevent evaporation. This provided a flexible seal that did not alter transscleral pressure. The temperature of the water-jacketed perfusion chamber was maintained at 37°C by a circulating water bath.

Permeability to three compounds was evaluated: 10^{-4} M 5(6) carboxyfluorescein (Eastman Kodak, Rochester, NY) diluted in BSS Plus, 2.37×10^{-6} M ^{3}H-dexamethasone-acetate (42.2 Ci/millimole; NEN, Boston, MA) packaged in ethanol and diluted to 10% (vol/vol) in BSS Plus, and ^{3}H-water (1 mCi/g; NEN) diluted to 10% (vol/vol) in BSS Plus. For experiments with carboxyfluorescein, the perfusate passed through a flow-through quartz cuvette (NSG Precision Cells, Farmingdale, NY), and measurements of total fluorescence in the cuvette were taken at 60-second intervals using a spectrofluorometer (Photon Technology, New Brunswick, NJ). Time-based fluorescence concentration was calculated using a standard dilution curve generated from a sample of the donor solution for each experiment.[7]

For the experiments with ^{3}H-water and ^{3}H-dexamethasone, samples of the perfusate were collected by a fraction collector (Isco, Lincoln, NE) at 10- to 15-minute intervals. At the completion of the perfusion, 50 μl of each fraction was added to 10 ml Aquasol (Packard, Meriden, CT), and tritium disintegrations were measured using a liquid scintillation counter (model LS 5801; Beckman, Irvine, CA). Disintegrations per minute (dpm) were calculated based on quenched standards of tritium. Samples (10 μl) were taken from the upper hemichamber at the beginning and end of the experiment to verify the initial donor concentration and to measure donor drug depletion over the course of the experiment.

Separate experiments were performed at transscleral pressures of 0, 15, 30, or 60 mm Hg. Pressure was applied across the tissue by raising the height of the outflow tube and was determined by measuring the distance between the tissue and the outflow tube as it flowed into the collector receptacle (e.g., 15 mm Hg was equivalent to a 22-cm water column). The pressure across the tissue was verified using a pressure transducer (Statham, Oxnard, CA) connected to the lower hemichamber.

Steady state permeability constant (K_{trans}) was calculated from the spectrofluorometry (carboxyfluorescein) or scintillation spectroscopy (^{3}H-water, ^{3}H-dexamethasone) data as:

$$K_{trans} = \frac{R_{total}}{(t)(A)} \times \frac{1}{[D]}$$

where R_{total} is the total amount of drug in the receiver effluent per collected fraction (measured as radioactive dpm or fluorescent counts), and t is the fraction collection time (in seconds). A is the area of exposed sclera (in square centimeters). This value—$R_{total}/(t)(A)$—is equal to the flux across the tissue. D is the concentration of drug in the donor hemichamber (dpm per cubic centimeter or counts per second per cubic centimeter). Permeability thus represents the steady state flux normalized by donor concentration. The area of exposed sclera was 0.385 cm^2 for the 7-mm chamber and 0.785 cm^2 for the 10-mm chamber.

Mean permeability values (±SD) were calculated from three to eight experiments performed for each compound at each pressure in both human and rabbit sclera. Analysis of variance (ANOVA) was calculated to compare the permeabilities at different pressures for each compound in both human and rabbit sclera. Tukey-Kramer multiple comparisons were then used to compare differences between pairs of pressure-dependent permeability measurements of each compound in human and rabbit sclera.

RESULTS

Human and rabbit scleral permeabilities to the compounds studied at different simulated intraocular pressures are shown in Figure 2 and Table 1. ANOVA showed human and rabbit scleral permeability to water (molecular weight: 18 Da, molecular radius: 2.0 Å) to be significantly affected by transscleral pressure ($P = 0.0004$ across human sclera and $P = 0.0039$ across rabbit sclera). The greatest difference in permeability was observed between 15 mm Hg and 30 mm Hg. The steady state K_{trans} of human sclera to water was measured as (mean ± SD) $5.18 \pm 1.85 \times 10^{-5}$ cm/sec at 15 mm Hg and $2.57 \pm 0.95 \times 10^{-5}$ cm/sec at 30 mm Hg. Rabbit scleral permeability to water was $5.43 \pm 1.28 \times 10^{-5}$ cm/sec at 15 mm Hg and $1.90 \pm 0.91 \times 10^{-5}$ cm/sec at 30 mm Hg. These differences represent a significant reduction in scleral permeability for both human and rabbit tissue at the higher pressure ($P < 0.01$),

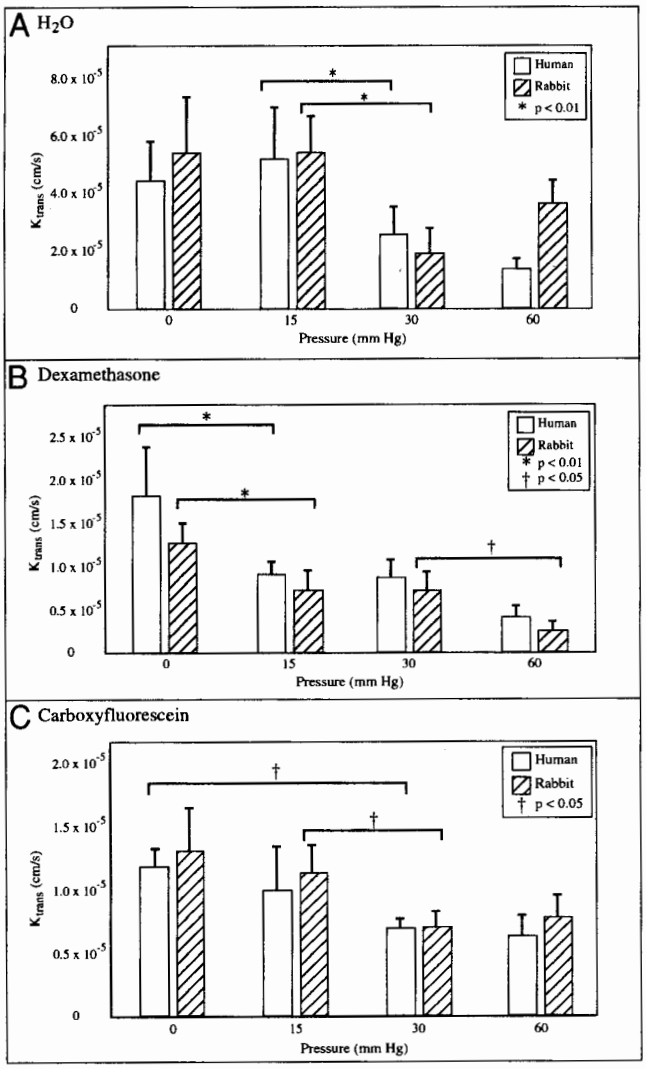

FIGURE 2. Permeability (mean ± SD) of human and rabbit sclera. (A) ^{3}H-water, (B) ^{3}H-dexamethasone, and (C) carboxyfluorescein at 0, 15, 30, and 60 mm Hg. Each histobar represents the mean ± SD of three to eight experiments (Table 1). *, †: statistical difference between pairs of data at a level of $P < 0.01$ and $P < 0.05$, respectively, determined by the Tukey-Kramer multiple comparisons test.

measured by the Tukey-Kramer test. Permeability was similar at 0 and 15 mm Hg for both tissues. Permeability was also similar at 30 and 60 mm Hg for both tissues. At 0, 15, and 30 mm Hg, human scleral permeability was similar to rabbit scleral permeability (see Fig. 2A). However, at 60 mm Hg, rabbit sclera was significantly more permeable to water than human sclera ($P = 0.002$; Student's t-test).

Scleral permeability to dexamethasone (molecular weight: 392 Da, molecular radius: 5.2 Å) was also significantly affected by transscleral pressure (Fig. 2B): $P < 0.0001$ with human tissue and $P = 0.0001$ with rabbit tissue (ANOVA). The greatest difference was observed between 0 and 15 mm Hg. At 0 mm Hg, permeability was measured at $1.82 \pm 0.58 \times 10^{-5}$ cm/sec across human sclera and $1.27 \pm 0.23 \times 10^{-5}$ cm/sec across rabbit sclera. Permeability to dexamethasone at 15 mm Hg was $8.94 \pm 1.5 \times 10^{-6}$ cm/sec and $7.12 \pm 2.3 \times 10^{-6}$ cm/sec for human and rabbit sclera, respectively. Scleral tissue was significantly less permeable to dexamethasone at 15 mm Hg than at 0 mm Hg ($P < 0.01$ for human and rabbit sclera; Tukey-Kramer test). There was also a significant decrease in permeability between 30 and 60 mm Hg for rabbit sclera ($P < 0.05$; Tukey-Kramer test). However, no difference was observed between 15 and 30 mm Hg in either species. Human scleral permeability to dexamethasone may be greater than rabbit scleral permeability to dexamethasone at comparable pressures ($0.08 < P < 0.25$; Tukey-Kramer test).

Figure 2C shows the permeability of human and rabbit sclera to carboxyfluorescein (molecular weight, 317 Da; molecular radius, 4.8 Å). The permeability of human and rabbit sclera to carboxyfluorescein was significantly affected by intraocular pressure ($P = 0.0064$ and 0.0016 for human and rabbit sclera, respectively; ANOVA). In general, permeability measurements between neighboring pressure values were similar, determined by the Tukey-Kramer test, with the exception of a difference in permeability across rabbit sclera at 15 and 30 mm Hg, when a significant decrease at the higher pressure was observed ($P < 0.05$). However, there was an overall trend of decreased permeability with increased pressure in both human and rabbit sclera. Moreover, scleral permeability at 60 mm Hg was significantly different from 0 mm Hg ($P < 0.05$ for human and $P < 0.01$ for rabbit sclera; Tukey-Kramer test).

TABLE 1. Permeability Constant (K_{trans}) for Water, Dexamethasone, and Carboxyfluorescein across Human and Rabbit Sclera

Transscleral Pressure	H_2O		Dexamethasone		Carboxyfluorescein	
	Human	Rabbit	Human	Rabbit	Human	Rabbit
0 mm Hg	44.6 ± 13 ($n = 8$)	54.4 ± 19 ($n = 5$)	18.2 ± 5.8 ($n = 6$)	12.7 ± 2.3 ($n = 5$)	11.8 ± 1.37 ($n = 4$)	13.0 ± 3.4 ($n = 4$)
15 mm Hg	51.8 ± 18 ($n = 7$)	54.3 ± 12 ($n = 5$)	8.94 ± 1.5 ($n = 5$)	7.12 ± 2.3 ($n = 4$)	9.93 ± 3.46 ($n = 6$)	11.4 ± 2.1 ($n = 5$)
30 mm Hg	25.7 ± 9.5 ($n = 6$)	19.0 ± 9.1 ($n = 4$)	8.65 ± 1.9 ($n = 5$)	7.07 ± 2.2 ($n = 5$)	6.81 ± 0.76 ($n = 4$)	6.97 ± 1.2 ($n = 4$)
60 mm Hg	14.0 ± 3.3 ($n = 4$)	36.7 ± 8.0 ($n = 4$)	3.92 ± 1.3 ($n = 6$)	2.33 ± 0.94 ($n = 3$)	6.15 ± 1.59 ($n = 5$)	7.66 ± 1.8 ($n = 7$)

Data are means ± SD × 10^{-6}. n represents number of eyes.

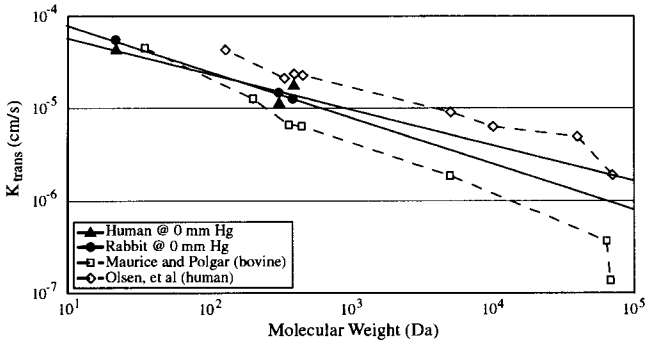

FIGURE 3. Comparison of human and rabbit scleral permeability. Measurements determined from the present study at 0 mm Hg were compared with those in two previous studies[2,3] The data of Maurice and Polgar[2] have been converted to permeability as described by Olsen et al.[3] The data collected from this study closely agree with previous work. However, note that lower scleral permeability occurred with increased intraocular pressure (Fig. 2).

DISCUSSION

This study shows that the sclera is permeable to low-molecular-weight compounds under the influence of a simulated intraocular pressure. Other studies have evaluated scleral permeability of different sized compounds without varying pressure. Data from the earlier studies are comparable to our data at 0 mm Hg (Fig. 3). Olsen et al.[3] measured the permeability of compounds ranging from 130 to 70,000 Da (radii ranging from 3.3 to 65 Å) across human sclera, and Maurice and Polgar[2] studied the diffusion of compounds from 23 to 69,000 Da (radii ranging from 1.0 to 64 Å) across bovine sclera. As shown in Figure 3, the zero-pressure permeability values measured in this study were comparable to those previously described. The current permeability values were slightly lower for human sclera than those in the previous report of human sclera. However, the chambers with constant mixing used in previous studies may yield a higher apparent permeability than that determined in the current study by using an unmixed depot on the surface of the sclera in which static boundary layers could form.

Although our results indicated that intraocular pressure could affect permeability, we found that the effect of pressure was small for the compounds tested. For all three compounds tested, the largest difference due to pressure was approximately a factor of two—that is, an increase of 15 mm Hg pressure causes scleral permeability to be reduced to approximately half its value at the lower pressure. Although intraocular pressure has a statistically significant effect on scleral permeability, it may not be sufficient to have clinical importance in a depot delivery system. In this study, the pressure was applied with the high pressure on the inner, choroidal side of the sclera and the low pressure on the outer, episcleral side where the drug solution was applied. For drug delivery into the eye, this is the physiologically correct orientation.

Elevated transscleral pressure could reduce rates of transport across sclera in two principal ways: It could induce flow of water across the sclera, which could carry molecules with it by convection, and it could directly reduce scleral permeability by altering the microanatomy of sclera. Considering the first mechanism, the role of water flow across the sclera can be addressed by comparing the rate of transport by flow-induced convection with that of diffusion. The Peclet number[8] (*Pe*) characterizes this:

$$Pe = \frac{v}{D/d}$$

where v is the velocity of the water flow, d is scleral thickness, and D is diffusivity in the sclera. The ratio of D/d gives a characteristic *velocity* of diffusion. Thus, the Peclet number can be thought of as the ratio of the velocity of convection to the velocity of diffusion. Assuming that the sclera is effectively homogeneous, which is consistent with its ultrastructure, then a Peclet number greater than 1 indicates that transport by pressure-induced flow is important. If it is much less than 1, then transport by flow is not important, and diffusion through the tissue should be the dominant mode of transport.

A representative value for the Peclet number can be calculated as follows. The sclera's hydrodynamic permeability is known to be 1.34 nm^2 at a pressure of 60 mm Hg and a temperature of 37°C.[8] This means that the velocity of water convection is $v = 2.6 \times 10^{-6}$ cm/sec. A characteristic human scleral thickness can be taken as $d = 0.4$ mm at 12 to 15 mm posterior to the limbus,[9] and effective diffusivity in the sclera for small molecules can be taken as $D = 1.1 \times 10^{-6}$ cm^2/sec.[10] Combining these values yields a diffusion velocity of 2.8×10^{-5} cm/sec, which is an order of magnitude greater than the velocity of water convection. This yields a Peclet number of $Pe = 0.095$.

Because the Peclet number is less than 1, the rate of transport by diffusion must be much greater than that by convection. This shows that water flow induced by high transscleral pressure is not sufficient to affect rates of diffusion across sclera significantly. As a result, increased pressure probably reduces scleral permeability by changing tissue microanatomy. This could be in the form of compressing the tissue, which in turn reduces the spaces between the collagen fibers and extracellular matrix molecules that define the pathways for diffusion. Smaller pathways hinder diffusion and thus lower scleral permeability. Measurements of tissue thickness and hydration as a function of pressure in recent experiments have verified this hypothesis (data not shown).

This report shows that scleral permeability to small compounds is a weak function of transscleral pressure. Past reports[2,3] have shown that scleral permeability, in the absence of an applied pressure, is a strong function of molecular weight. It is likely that these two effects are synergistic, rather than simply additive, when diffusion of macromolecules in the presence of a transscleral pressure is considered. Because the Peclet number analysis indicates that pressure reduces scleral permeability by compressing fibers (e.g., collagen, extracellular matrix) within the sclera, narrowing intracollagen pathways should affect diffusion of macromolecules more than small molecules because of the molecular size (i.e., nanometers) of the pathways.[10] Thus a narrowing of intramolecular fibers within the sclera slows diffusion of small molecules yet may completely block transport of macromolecules. Future studies are needed to test this hypothesis. Although the permeability of the sclera to larger compounds as a function of pressure has not yet been determined, the data presented in this report indicate that intraocular pressure is an important consideration in developing a practical model of drug delivery across the sclera.

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