Anatomical and Physiological Studies on the Aqueous Sinus Artery of the Pigeon Eye

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Purpose: To test the hypothesis that ischemia of the Schlemm’s canal endothelium is involved in the control of intraocular pressure (IOP).

Methods: Experiments were conducted on pigeons. The course of the aqueous sinus and the aqueous sinus artery was determined by examining serial sections and the distribution of polymerized resin in the limbal region of the pigeon eye. Ischemia was produced by blocking two major arteries to the aqueous sinus artery and then determining the effect on the IOP.

Results: The anatomy and course of the pigeon aqueous sinus and aqueous sinus artery indicated that they are homologous to the human Schlemm’s canal and Schlemm’s canal artery, respectively. When the arterial inflow to the aqueous sinus artery was blocked by laser treatment, injected resin was absent in the artery but a small amount invaded the aqueous sinus. Blockage of the two major arteries to the aqueous sinus artery did not alter the IOP.

Conclusions: The similarity of the pigeon aqueous sinus and aqueous sinus artery to the human Schlemm’s canal and Schlemm’s canal artery indicates that the pigeon eye can be used to study the relationship between ischemia of the endothelial meshwork cells and IOP. The lack of a change in IOP in pigeons after ischemia induction was probably the result of anastomotic arterial blood inflow to the aqueous sinus directly, and thus ischemia was not produced by blocking the two major arteries to the aqueous sinus artery. Jpn J Ophthalmol 1999;43:262–271 © 1999 Japanese Ophthalmological Society

Key Words: Aqueous sinus, aqueous sinus artery, ischemic theory, open-angle glaucoma etiology, pigeon eye.

Introduction

We have shown previously that severing all of the anterior ciliary arteries induced a transient ocular hypertension in monkeys and suggested that the blockage of blood flow to the endothelial cells of Schlemm’s canal altered their physiology, which in turn upset the movement of fluid out of the eye. This altered outflow then resulted in an increased intraocular pressure (IOP). These observations supported our hypothesis first published in 1981, that open-angle glaucoma of human eyes is caused by ischemia of the cells of Schlemm’s canal.

In the human eye, a small artery runs in the outer wall of Schlemm’s canal, not completely circumferentially, but for a considerable distance. The artery, called Schlemm’s canal artery, is a branch of the penetrating anterior ciliary artery and branches in either the sclera or the episclera. We proposed that blockage of Schlemm’s canal artery, which nourishes the cells of the endothelium, would alter the physiology of these cells and thus alter the movement of fluid out of the eye.

To obtain additional data to support our ischemia hypothesis, a search was made for an animal more readily available than monkeys, whose vascular anatomy of the anterior segment of the eye is similar to that of the human eye. In general, mammalian eyes are not equipped with a distinct vessel like Schlemm’s canal for aqueous drainage, except in the higher primates, Simiae. However, the avian eye has a distinct trunk vessel for aqueous drainage that is called the aqueous sinus. The aqueous sinus is considered to be homologous to the Schlemm’s canal of the human eye.
higher primates. Just as the Schlemm’s canal artery runs in the outer wall of Schlemm’s canal, the aqueous sinus artery in pigeons is very large and is situated in the lumen of the aqueous sinus.\textsuperscript{5–8} Thus, the pigeon aqueous sinus artery appears to be analogous to Schlemm’s canal artery of the human eye.

The purpose of this study was to determine whether the pigeon eye can be used to study the factors that control the IOP of the eye, and thus be used to obtain evidence for the ischemia hypothesis. To accomplish this, it was necessary to determine the course of the pigeon aqueous sinus artery and aqueous sinus and compare them to the course of Schlemm’s canal and Schlemm’s canal artery. This study shows that the aqueous sinus and aqueous sinus artery are morphologically homologous to Schlemm’s canal and Schlemm’s canal artery and concludes that the pigeon can be used to study the ischemia hypothesis. To test the ischemia hypothesis in pigeons, this study attempted to induce ischemia by blocking the two major inflow routes to the aqueous sinus artery and measure the IOP before and after the blockage.

**Materials and Methods**

**Animals**

The experiments were conducted on white pigeons, *Streptopelia risoria*, of both sexes and weighing 130–180 grams each. The pigeons were obtained from a local vendor (Sakura Co., Aichi). They were maintained in the laboratory and given free access to avian chow (Ishibashi Co., Osaka) and drinking water. This study was conducted in accordance with the ARVO resolution on the use of animals in research.

**Anatomical Studies**

To determine the normal morphology of the aqueous sinus and the aqueous sinus artery, serial sections of the anterior segment of the eye were made using the 26 eyes of 13 pigeons. The animals were killed with an intramuscular injection of 50 mg of ketamine hydrochloride (Ketalar\textsuperscript{™}; Sankyo, Tokyo), and the anterior segments were removed by cutting the sclera circumferentially with a scalpel and a pair of scissors. For tissue orientation, a hole was made on the edge of the segment facing the animal’s bill. The anterior segments were immersed immediately in 10% formalin and the central part of the lens was “cut out” by making a circumferential series of holes with a 30-gauge needle that penetrated from the lens to the cornea. A photograph was taken of the remaining portion that included the periphery of the lens, called the annular pad. After 24 hours of fixation, each segment was dissected radially (meridionally) into eight pieces. The pieces were dehydrated and embedded in hydrophilic resin (Technovit 7100\textsuperscript{™}; Kulzer, Wehrheim, Germany). Meridional, 7-\mu m-thick serial sections were cut with a microtome (HM350; Microm, Heidelberg, Germany) and stained with 0.1% toluidine blue. The stained sections were examined by light microscopy.

To determine the relationship between the aqueous sinus and aqueous sinus artery to the IOP, we studied the aqueous sinus and aqueous sinus artery in eyes in which the IOP was reduced in vivo. Five eyes of five pigeons were used with the contralateral eyes as controls. To accomplish this, the animal was anesthetized by an intramuscular injection of 15 mg ketamine, and the lids were spread apart by a lid retractor without exerting pressure on the globe (Figure 1). An incision was made through the cornea to reduce the IOP, and the animal was then killed by an additional intramuscular injection of ketamine. To insure rapid exposure of the anterior segment to the fixative, the lid retractor was removed, the lower lid was excised, and then the head was immediately immersed in a 10% acrolein solution for 1 hour. The anterior segments were then excised and further fixed in 10% formalin solution for 24 hours. The lens was removed intact, and the ciliary body and iris were peeled off along the deep ciliary cleft with forceps. These segments were studied in formalin solution with a stereomicroscope.

Another technique used to study the anatomy of the aqueous sinus and aqueous sinus artery was to make a cast of the aqueous sinus artery network in the 32 eyes of 16 pigeons. To accomplish this, the an-

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*Figure 1. Handmade applanators and lid retractor.*
Animals were killed by an overdose of ketamine and 20 mL of a casting resin monomer (Mercox™; Dai Nippon Ink, Tokyo) mixed with 0.2 mL of polymerizing agent was injected into the left ventricle of the heart. After polymerization (15–60 min), the anterior segments of the eyes were removed as described and immersed into an α-chymotrypsin (Zonolysin™, Mochida, Tokyo) solution of 125 NFU/mL at 37°C for 20 hours to remove the lens and retinal pigment epithelium (RPE). After 3 hours of supplemental fixation in 10% formalin, the anterior segments were cleared in glycerol at 4°C for 48 hours. The resin-filled vessels were then examined in relation to the other tissues using a stereomicroscope.

Ischemia and IOP

To test the ischemia hypothesis, the following five procedures were performed consecutively on six pigeons, with three pigeons treated in the right eye and three in the left eye.

Measurement of the control IOP. The pneumotonomograph (PTG) tonometer (Alcon, Fort Worth, Texas, USA) was selected to measure the IOP of the eyes because measurement of the IOP by cannulating the anterior chamber did not give reproducible results. This was due to the thinness of the pigeon cornea, which could not support the cannulating needle (30-gauge). In preliminary studies with the PTG tonometer, we found that the IOP decreased considerably after placing the applanators (described below) on the cornea, and a 10-minute recovery period was required for the IOP to return to the preapplanation level. Therefore, tonometry was repeated only after a 20-minute recovery period.

To record the IOP without using a lid retractor, the bill of the pigeon was held by one hand, and a drop of 0.4% oxybuprocain (Benoxyl™; Santen, Osaka) was instilled. The lower lid was pushed aside by the tip of the sensor held in the other hand, and the IOP was recorded by placing the tip of the sensor on the cornea.

Rupturing Anastomotic Artery by YAG Laser. After the measurement of the control IOPs, a plastic plate was placed between the bill to maintain a clear breathing passage, the bill was then fixed by pincers. The body was placed in the supine position, and a drop of oxybuprocain was instilled. To examine the lower chamber angle, an applanator, made of a piece of microscope slide and an 18-gauge hypodermic needle (Figure 1), was placed on the upper section of the cornea. The ciliary cleft was identified through the applanator with a slit-lamp microscope equipped with a YAG laser (Nidek, Aichi) (Figure 2). The anastomotic artery and accompanying white bridge were found crossing over the ciliary cleft (Figure 3A).

If the anastomotic artery could not be found, the body was returned to its normal position, and a strong light from a 75 W halogen lamp was projected through a fiber optic light guide into the contralateral eye. With this illumination, the pupil of the experimental eye had a reddish glow, and the “navel” (see below) was easily seen through the sclera (Figure 3B).

Returning the animal to the supine position, the anastomotic artery was found by using the navel and ciliary cleft as landmarks. The anastomotic artery was then exposed to 3.4 mJ of the YAG laser. The appearance of bleeding was a sign that sufficient treatment had been given.

Coagulation of penetrating artery by DYE laser. Returning the bird to the normal position, additional anesthetic eyedrops were instilled on the same eye, and another applanator, which was constructed of an acrylate plate and a needle (Figure 1), was placed on the lower limbus of the same eye. This 3-mm-thick applanator was sufficient to keep the lower lid open. To distinguish arteries from veins, the applanator was pressed gently on the limbus to collapse the veins, whereas the arteries remained patent. One exposure of the DYE laser (Nidek) at 595 nm, 0.2-mm diameter, at 1.4 W, and for 0.3 seconds, whitened the artery; but additional shots, at least 10, were given along the artery to insure occlusion of the penetrating artery. After the treatment with the YAG and DYE laser, the restraints on the bird were removed.

Figure 2. View of lower chamber angle.
and the animal was free to move about and obtain water.

**Measurements of IOP.** Twenty minutes after the laser treatments, the IOP of the treated and control eyes was measured every 20 min for 2 hours as described above.

**Injection of casting resin.** After the last IOP measurement at 2 hours, the animal was given an overdose of ketamine. Immediately after death, the thorax was opened and 20 mL of casting resin with polymerizing agent was injected into the left ventricle as described. After the resin polymerized, the anterior segments were dissected out and the central part of the lens removed. Photographs were then taken, and the anterior segments were fixed for 24 hours. The anterior segments were prepared for serial sectioning as described.

**Results**

**Normal Morphology of the Aqueous Sinus and Aqueous Sinus Artery**

Three sets of blood vessels were found on the external surface of the anterior segment. The vessels ran into the conjunctiva from the lower, upper-anterior, and upper-posterior fornix, and each set of vessels consisted of one artery placed between two veins. The vessels fan out as they approach the limbus. The artery of the lower set of vessels penetrates the sclera at the limbus, is branched very little, and is called the penetrating artery.

On the internal surface of the anterior segment, a pigmented fleck was found on the lower part of the inner surface of the ciliary region (Figure 4A). The center of the fleck was depressed and will be referred to as the “navel” because of its appearance.

In serial sections, one or two bony plates, called ossiculi (usually cracked during sectioning), were found in the sclera (Figure 4B, 4C). The aqueous sinus was identified on the corneal side of the ossicula as a crescent-shaped hole. The large aqueous si-

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**Figure 3.** (A) Internal view of lower chamber angle. (Large arrow: aqueous sinus artery; small arrow: anastomotic artery and accompanying white bridge.) (B) View of eye transilluminated from light directed into contralateral eye. (Arrow: “navel.”) (C) Laser-treated anastomotic artery after resin injection. (Small arrow: broken anastomotic artery; large arrow: bridge; white arrow: iris artery filled with resin.) (×78) (D) Laser-treated penetrating artery after resin injection. (Arrow: burned penetrating artery; white arrow: iris artery filled with resin.) (×78)
nus artery was found in the lumen of the aqueous sinus and divided the lumen into two sections (Figure 5, left panel). Each section was connected to many aqueous draining paths that ran through the sclera near the cornea in front of the ossicula. These paths merged into the subconjunctival veins. In serial sections of the lower-anterior region of the anterior segment near the bill, the aqueous sinus was smaller and sometimes not found at all. However, the aqueous sinus artery was found in all sections of the eight blocks and was larger in the lower than in the upper half of the anterior segment. There were several small branches from the aqueous sinus artery toward the sclera but never toward the cornea (Figure 5, fifth panel on left).

A distinct morphological feature of the pigeon eye was the two junctions made by the aqueous sinus artery with the two inflow arteries in the sections obtained from the lower polar area. One of these junctions was an anastomotic junction with the iris artery that crossed over the ciliary cleft (Figure 4B). This anastomotic artery was accompanied by connective tissue that formed a bridge between the sclera and the iris over the cleft. The ciliary region at this position formed the “navel” (Figure 4A). At the other junction, the aqueous sinus artery merged with the penetrating artery that came from the lower limbus (Figure 4C). These junctions were situated near each other and, in all cases, the junction with the anastomotic artery, which forms the navel, was located more anteriorly (toward the bill) than the junction with the penetrating artery (Figure 6).

In a few specimens, a small, tortuous artery was found that merged into the aqueous sinus artery and appeared as an appendix in the upper or posterior part. An appendix to the anastomotic artery was never found.

**Physical Effect of the IOP on the Aqueous Sinus**

When the IOP was reduced by the corneal incision, venous blood flowed retrogradely into the aqueous sinus from downstream veins. The blood,
Figure 5. Resin distribution after unilateral laser treatment. (Small arrow: aqueous sinus artery [ossiculum in left corner shows right eye and in right corner shows left eye]; large arrow: resin.) (×49)
fixed by the rapid acrolein fixative, was trapped in the aqueous sinus (Figure 4D). This view showed that the lumen of the aqueous sinus was smaller or sometimes not present in the vicinity of the bill. There were also many drainage paths from the aqueous sinus (Figure 4D).

**Examination of Casts of the Aqueous Sinus Artery**

The polymerized resin traced the course of the arteries running through the anterior segment (Figure 6). The ossicula, which overlapped and roughly delineated the corneoscleral border, were visible through the semitransparent sclera in the glycerol-cleared preparation. The sphincteric capillary plexus, which is involved in accommodation, appeared as a dark ring in the iris. The upper and lower ciliary arteries merged with the plexus at each of two points.

The aqueous sinus artery formed an apparently complete circle although the aqueous sinus was sometimes absent in the vicinity of the bill. In the lower part of the aqueous sinus artery circle, the two large junctions found in the serial sections were identified, the junction of the aqueous sinus artery with the anastomotic artery and the junction with the penetrating artery.

**Effect of the Blockage of the Input to the Aqueous Sinus Artery on the IOP**

Both the laser-treated and the untreated contralateral eyes maintained the same IOP of 24–26 mm Hg as before treatment for as long as 2 hours after the laser treatments (Table 1). Thus, there was no significant change in the IOP after the laser treatments.

**The Aqueous Sinus in the Lower Polar Region After Laser Treatments**

Serial sections of the untreated contralateral eye showed that resin filled the entire aqueous sinus artery, the penetrating artery, the anastomotic artery, and the iris arteries. In the treated eye, the ruptured and deformed anastomotic artery was found in the region of the YAG laser burn (Figure 3C). Evidence of bleeding was seen in the anterior chamber. In the region of the DYE laser coagulation, a blood coagulum was found in the penetrating artery (Figure 3D). The presence of the coagulated blood 2 hours after the treatment demonstrated that the arterial blood flow had not been restored. In both areas, resin filled only the iris arteries (Figure 3C, 3D).

**The Aqueous Sinus After Laser Treatments (Except for the Lower Polar Region)**

In the untreated eye, the entire aqueous sinus artery and ciliary artery were filled with resin (Figure 5, left panels). The small tortuous appendix artery...
did not fill with resin most likely because of its size. In the treated eye, on the other hand, the aqueous sinus artery was thin and had no resin throughout, indicating that the arterial flow was completely blocked to this vessel (Figure 5, right panels). Surprisingly, resin was found in the aqueous sinus itself in either the upper or posterior part for 10%–30% of its length. In a few sections, a piece of resin was also found along the aqueous draining path that had horn-like projections (Figure 5, right side, fifth panel). A careful tracing in successive sections did not reveal any connections of these projections to any artery. In addition, the resin in the aqueous sinus also did not show a connection to the episcleral arteries. Thus, we cannot presently explain whether the horn-like projections of a piece of resin represent inflow to or outflow from the aqueous sinus.

The aqueous sinus of the treated eyes appeared very deformed when compared with the aqueous sinus of the contralateral eyes. This was partly due to the shrinkage of aqueous sinus artery induced by the ischemia and partly due to the outward pressure of the resin. These sections also showed that the position of the aqueous sinus artery in the aqueous sinus lumen was not fixed in the middle. These findings were found uniformly in all of the treated and non-treated eyes of all six pigeons from the ischemia test.

### Discussion

**IOP Measurements of the Pigeon Eye**

To the best of our knowledge, the intraocular pressure of the pigeon eye has not been published; although the pressure of the chicken eye was measured by the cannulation method. The IOP of the chicken eye was 15 mm Hg and was not affected by intravenous acetazolamide.\(^\text{10}\) The PTG tonometer, designed for human eyes, gave reproducible values for the pigeon eye, although it measured only the relative IOP. Repeated measurements of the IOP showed the so-called “tonography effect,” ie, repeated determinations lowered the IOP significantly. Therefore, only a minimal number of measurements were made.

It is known that bleeding into the anterior chamber leads to an increase in the IOP in the human eye. In the pigeon, however, I found that bleeding into the anterior chamber after YAG laser treatment did not alter the IOP significantly or affect the return of the IOP to the preapplanator application level, ie, the IOP recovered relatively quickly and never exceeded the level before the treatments. This suggests that the blood coagulation mechanism is very rapid in the pigeon, and hemostasis is completed even before removing the applanators.

### Table 1. Course of IOP (mm Hg) After Laser Treatment

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Average and Standard Deviation in IOP in Treated Eyes

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Average and Standard Deviation in IOP in Control Eyes

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*: Treated eyes.
Our observations showed that the IOP of the unanesthetized pigeon eye was 24–26 mm Hg by my technique and apparatus. Blockage of blood flow to the aqueous sinus artery by laser treatments of the anastomotic and penetrating artery did not alter the IOP of the eye. Thus, these findings do not support our ischemia hypothesis for ocular hypertension. However, as shown, resin was found in the aqueous sinus of the treated eye. Therefore, complete ischemia had not been produced. Further experiments will be needed to produce complete ischemia of the aqueous sinus to obtain a definitive answer to our hypothesis.

Inflow Into Aqueous Sinus

Both the aqueous sinus and Schlemm’s canal are blind sacks opening only to veins, and the IOP is very important in controlling what flows into them. When the IOP is low, the inflow is very smooth because the trabecular meshwork moves toward the anterior chamber as discussed previously. Figure 4D shows that venous blood flowed retrogradely into the aqueous sinus after a decrease in IOP resulting from venous pressure as a physical phenomenon. When resin was injected into the heart immediately after death, without laser treatment, resin was never found in the aqueous sinus. Occasionally, it was found macroscopically only in the very large trunk vein. Other laboratories using the casting resin technique reported on the vascular cast of the duckling eye. In their method, the animal was first bled and then perfused with Ringer’s solution before injection of the resin. Based on their observations, we chose to inject the resin: (a) immediately after death when the IOP was still maintained, (b) without prior bleeding and washing out of the blood, (c) only in small quantity (20 mL), (d) using the same conditions for both eyes, and (e) comparing both eyes post factum. Under these conditions, retrograde flow of the resin from a trunk vein into the aqueous sinus should not be possible. As shown, resin invaded the upper and posterior parts but not the lower polar region of the aqueous sinus in the treated eye after treatment by YAG and DYE laser. The YAG-ruptured anastomotic artery did not leak resin (Figure 3C). Moreover, resin was never found in the region of the YAG-ruptured point or the DYE-burned point except for the iris artery (Figure 3C, 3D). These findings were as expected, because resin could not flow into these regions without blood flow.

From these observations, we suggest that the resin invaded the aqueous sinus in the treated eyes through some anastomotic routes, viz, an arterio-sinus or arterio-venous anastomosis, which might function under ischemic conditions. The two arteries of the three sets of vessels on the front surface of anterior segment, but not the penetrating artery of the lower set, may be involved in the collateral arterial route. Similar arterio-venous anastomoses have been reported in the episclera of dogs and monkeys, and the search for these anastomoses in pigeons is being undertaken. The small tortuous appendix artery found in the serial sections, never filled by resin perhaps because of the viscosity of the resin, may have a similar function, although it is an arterio-arterial (A-A) anastomosis.

Relationship to the Previous Study on Monkey Eyes

As stated, we succeeded previously in inducing a transient ocular hypertension in monkeys by severing all anterior ciliary arteries that induced ischemia of Schlemm’s canal. These results supported our hypothesis that open-angle glaucoma in the human eye is caused by ischemia of the endothelium of Schlemm’s canal. However, in the monkey eye the distance between the severed points and Schlemm’s canal was large, and the possibility existed that Schlemm’s canal was nourished by the long posterior ciliary artery via the major arterial circle and the rest of the anterior ciliary artery. To produce an absolute ischemia of the endothelium of the Schlemm’s canal, blockage of the arterial inputs should be made close to the canal. This was the rationale for the site of the lesions in the pigeon eyes, ie, treating the aqueous sinus artery near the junction with the two inflow arteries. If ischemia of the endothelium of Schlemm’s canal is the underlying mechanism for the rise in IOP, there should have been a rise in the IOP after the laser treatments of the penetrating and anastomotic arteries in pigeons. As shown, the IOP did not rise but the experiments proved to be inconclusive as resin was found in the aqueous sinus indicating that complete ischemia was not produced. In retrospect, an upstream occlusion of arterial blood may have been more effective in inducing ischemia of the aqueous sinus because it should also eliminate anastomotic collateral routes.

The author thanks Kenichi Kishida, MD, PhD for his generous support.
References


