The effect of complete tenotomy on blood flow to the anterior segment of the canine eye

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The relative importance of the anterior ciliary arteries to blood flow to the anterior segment and the quadrant distribution of blood to the iris, ciliary body, and ciliary processes were determined in canines with the use of 15 ± 3 μm 153Ru microspheres with the reference blood flow method. Recti muscles were isolated in both eyes. Then with one eye serving as a sham-operated control, anterior ciliary artery blood flow was disrupted by recti tenotomy immediately prior to microsphere injection. Tenotomy resulted in a significant decrease in blood flow to the anterior segment (0.14 ± 0.03 ml/min/gm vs. 0.26 ± 0.05 in the untenotomized control) and to the unit iris, ciliary body and ciliary processes (I, CB, CP) (0.92 ± 0.16 ml/min/gm vs. 1.44 ± 0.22 in the untenotomized control). Blood flow values for the anterior segment and the unit I, CB, CP in the tenotomized eye were 50% to 60% of those of the control eyes, indicating that the majority of blood flow to the canine anterior segment is not disrupted by complete tenotomy. In the control eye, blood flow values for the medial quadrant of the unit I, CB, CP were significantly higher (p < 0.05) than those of the inferior or superior quadrants. Blood flow values for all quadrants in tenotomized eyes were 55% to 70% of those for control eyes. Therefore the anterior ciliary arteries do not contribute the majority of blood flow to the canine anterior segment.

Key words: tenotomy, blood flow, anterior segment, canine eye

Two major arterial circulations provide the vascular supply to the anterior segment (AS) of the mammalian eye: (1) the anterior ciliary arteries (ACA), which are continuations of the muscular branches of the ophthalmic artery, and (2) the long posterior ciliary arteries (LPCA), which originate near the optic nerve.1–3 The LPCA pass forward in the horizontal meridian and divide into two main branches at the outer aspect of the ciliary body. These branches then anastomose with the ACA forming the major circle of the iris.1 Although the anatomic relationships between the LPCA and the ACA are well known in a number of species,4,5 few investigators have correlated anatomy with function. The physiologic relationship between the ACA and the LPCA, as well as their relative importance to AS blood flow, is critical to the understanding of such clinical problems as AS ischemia following strabismus or retinal detachment surgery.

Recently, we determined the relative importance of the anterior ciliary arteries to blood flow to the anterior segment and the quadrant distribution of blood to the iris, ciliary body, and ciliary processes in canines with the use of 15 ± 3 μm 153Ru microspheres with the reference blood flow method. Recti muscles were isolated in both eyes. Then with one eye serving as a sham-operated control, anterior ciliary artery blood flow was disrupted by recti tenotomy immediately prior to microsphere injection. Tenotomy resulted in a significant decrease in blood flow to the anterior segment (0.14 ± 0.03 ml/min/gm vs. 0.26 ± 0.05 in the untenotomized control) and to the unit iris, ciliary body and ciliary processes (I, CB, CP) (0.92 ± 0.16 ml/min/gm vs. 1.44 ± 0.22 in the untenotomized control). Blood flow values for the anterior segment and the unit I, CB, CP in the tenotomized eye were 50% to 60% of those of the control eyes, indicating that the majority of blood flow to the canine anterior segment is not disrupted by complete tenotomy. In the control eye, blood flow values for the medial quadrant of the unit I, CB, CP were significantly higher (p < 0.05) than those of the inferior or superior quadrants. Blood flow values for all quadrants in tenotomized eyes were 55% to 70% of those for control eyes. Therefore the anterior ciliary arteries do not contribute the majority of blood flow to the canine anterior segment.
Tenotomy

SR
MR
IR
LR

for counting (1)

Control

Fig. 1. Schema of the recti (superior, SR; inferior, IR; medial, MR; lateral, LR) and the corresponding quadrants of the AS (circle). Recti are clamped and tenotomized at the insertion (striped line) in the experimental eye.

The importance of the ACA to primate AS blood flow. This present study was designed to determine whether the relative contribution of the ACA was similar in all mammals. A second aim of the study was to establish the distribution of blood flow to the ocular quadrants of the ACA before and after tenotomized disruption of the circulation of the ACA. The ocular quadrants considered for AS blood flow were units of one fourth of the tissue of the iris, ciliary body, and ciliary processes (I, CB, CP) oriented radially at the individual rectus insertion (see Fig. 1).

The canine was chosen as our experimental animal because although the dog has been used as a model for AS ischemia, the physiology of AS circulation has not been studied. Additionally, the dog has been used extensively in cardiovascular research, and although its vascular physiology is well known (see refs. 8 and 9) ocular blood flow values have not been determined.

Materials and methods

Animals. Six adult male mongrel dogs (24 to 29 kg bw) were anesthetized with Nembutal (50 mg/ml). Animals were left under continuous cardiovascular monitoring for the duration of the experiment. Catheters were placed in the pulmonary artery (Swan-Ganz), femoral artery and vein, and left ventricle. Direct arterial pressures, left ventricular pressures, and cardiac outputs were monitored. The pulse, blood pressures, and electrocardiogram (EKG) were continuously recorded. Cardiac outputs were determined by thermal dilution after catheterization, before and after muscle isolation, before and immediately after injection of microspheres, and before sacrifice.

Microspheres. A 3 to 3.5 ml injection dose of NEN-Trac $^{103}$Ru ($15 \pm 3 \mu m$ suspended in saline with 0.1% Tween 80) containing 13.5 to 15.75 x $10^6$ spheres was used. The dose was drawn with a Rainin Pipetman and agitated in a disposable syringe until delivery via the left ventricular catheter. The dose was delivered over a 10 to 15 sec period, and the syringe was flushed with blood and then saline.

Experimental. Each animal had both eyes peritomized 360°; the conjunctiva was separated cleanly from all underlying tissues, and all recti muscles in both eyes were isolated by standard strabismus surgery. Care was taken to separate all facial tissues in isolating the recti down to bare sclera. The animal was allowed to stabilize for 30 min. The recti in the experimental eye were clamped and tenotomized at their insertion, and the microspheres were immediately injected. A 1 min reference blood flow collection from the femoral artery was initiated simultaneously with the start of the microsphere injection. At 4 min after injection, a venous blood sample was collected to check for recirculation of spheres. Five minutes after injection the animal was sacrificed by an intracardiac injection of KCl; four quadrants of each eye were marked, eyes were enucleated, and the kidney was removed.

Processing and analysis. All samples were weighed immediately after removal and fixed in 4% buffered formalin for 48 hr. Samples were then reweighed, and eyes were dissected first at the ora ciliaris into anterior and posterior segments and counted for radioactivity. The posterior segment was then further separated into choroid, retina, posterior sclera, and vitreous; the AS was dissected into four separate quadrants. All samples were counted with a sodium iodide single-channel well-type detector (480 to 660 KeV window). Subsequently the four AS quadrants were further divided into total sclera/cornea segment and four quadrants of the unit I, CB, CP and counted (see Fig. 1). The counts per minute (cpm) were converted into blood flow values with the reference blood flow method and calculated by the equation:

$$\text{Blood flow to tissue (ml/min)} = \frac{\text{cpm tissue} \times \text{gm/min reference blood flow}}{\text{cpm reference blood} \times \text{specific gravity of blood gm/ml}}$$
Tenotomy and blood flow to anterior segment

Results

No differences were noted between wet weight and 48 hr fixed weight. Therefore all blood flow determinations (milliliters per minute per gram) were based on fixed tissue weights.11

More than the minimum number of spheres (400) needed for statistical accuracy12 were impacted in each eye segment studied. The dose of microspheres injected did not cause any hemodynamic effects, as evidenced by lack of significant change in blood pressure, pulse, EKG, and especially cardiac output during the course of the experiment. The number of spheres found in the venous sample indicated that there was no significant amount of recirculation.

The insertion of the retractor bulbi was consistently near or behind the equator. In a few exceptions, part of the retractor insertion came forward between the lateral and superior recti or the lateral and inferior recti, so that it could have contributed to the AS blood flow. In these exceptional cases all muscles approaching the AS were tenotomized, thereby completing a total devascularization of the AS from extrinsic ocular circulation.

The control eye had a mean blood flow of 2.29 ± 0.34 ml/min (0.33 ± 0.05 ml/min/gm), and the mean blood flow ratio (choroid/iris, ciliary body and ciliary processes) was 5.2 ± 0.81. Tenotomy resulted in a significant decrease in blood flow to the AS (p < 0.05), the unit I,CB,CP (p < 0.01), and the anterior sclera (p < 0.025) (Table I). Blood flow in the tenotomized eye (1) to unit I,CB,CP was approximately 63% that of controls, (2) to the anterior sclera was approximately 28% that of controls, and (3) to the entire AS was approximately 53% that of controls.

In the control eye, blood flow values for the medial quadrant of the unit I,CB,CP were significantly higher (p < 0.05) than those for the superior or inferior quadrants (Table II). Blood flow values for all quadrants in tenotomized eyes were significantly less than those for control eyes (Table II). No significant differences in the percent of blood flow reduction due to tenotomy were found among the quadrants.

Discussion

Although the dog has been used as an experimental model for AS ischemia,7 the canine orbital and ocular vasculature differs from that found in human and nonhuman primates. Unlike primates, the dog possesses an incomplete bony orbital floor, a vascularized nictitating membrane, and a retractor bulbi muscle, which appears as either a complete muscle cone or divided into four parts.8 In dogs the main blood supply to the orbit and globe is provided by an external

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**Table I.** Blood flow values (ml/min/gm) for the canine eye (mean ± S.E.M.; no. of eyes = 12)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Tenotomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye</td>
<td>0.33 ± 0.05</td>
<td>0.26 ± 0.07</td>
</tr>
<tr>
<td>Posterior segment</td>
<td>0.48 ± 0.10</td>
<td>0.46 ± 0.12</td>
</tr>
<tr>
<td>AS</td>
<td>0.26 ± 0.05</td>
<td>0.14 ± 0.03</td>
</tr>
<tr>
<td>Choroid</td>
<td>9.61 ± 2.08</td>
<td>10.03 ± 2.03</td>
</tr>
<tr>
<td>Retina</td>
<td>0.12 ± 0.07</td>
<td>0.12 ± 0.06</td>
</tr>
<tr>
<td>Sclera from AS</td>
<td>0.21 ± 0.05</td>
<td>0.06 ± 0.01</td>
</tr>
<tr>
<td>Sclera from posterior segment</td>
<td>0.25 ± 0.07</td>
<td>0.26 ± 0.06</td>
</tr>
<tr>
<td>I,CB,CP</td>
<td>1.44 ± 0.22</td>
<td>0.92 ± 0.16*</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.92 ± 0.10</td>
<td></td>
</tr>
</tbody>
</table>

Significantly different from control by t test for paired comparisons: *p < 0.01; 1p < 0.025; or 2p < 0.05.

**Table II.** Blood flow values (ml/min/gm) for quadrants of the unit I,CB,CP (mean ± S.E.M.; no. of eyes = 12)

<table>
<thead>
<tr>
<th>Quadrants</th>
<th>Control</th>
<th>Tenotomy</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superior</td>
<td>1.09 ± 0.14</td>
<td>0.70 ± 0.18</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Inferior</td>
<td>1.26 ± 0.22</td>
<td>0.99 ± 0.19</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Medial</td>
<td>1.64 ± 0.25</td>
<td>0.99 ± 0.15</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Lateral</td>
<td>1.52 ± 0.32</td>
<td>0.83 ± 0.19</td>
<td>&lt;0.025</td>
</tr>
</tbody>
</table>

*p values are the probabilities that blood flow to a quadrant in the tenotomized eye is significantly different from blood flow to the corresponding quadrant in the control eye as determined by a t test for paired comparisons.

Values for the lateral quadrant were less than those for the medial quadrant, but the difference was not significant (p < 0.20) (Table II). Blood flow values for all quadrants in tenotomized eyes were significantly less than those for control eyes (Table II). No significant differences in the percent of blood flow reduction due to tenotomy were found among the quadrants.
ophthalmic artery derived ultimately from the external carotid. The internal ophthalmic enters the orbit to anastomose with the ciliary branch of the external ophthalmic. The conjunctiva/facial tissues anterior to the recti insertions are more vascularized than in primates.

The blood flow ratio (defined by choroid/1,CB,CP) gives a quantitative estimate of blood flow distribution between the posterior and anterior segments of the eye. This ratio has been determined as 8:1 to 11:1 in primates. A much lower ratio was found in this study for canines, indicating a relative increase in the proportion of ocular blood flow to the AS.

The present study demonstrates that although tenotomy reduces the blood supply to the canine AS, 50% to 60% of the blood supply is undisturbed by disruption of the ACA. Therefore, in contrast to primates, the ACA do not contribute the majority of blood flow to the canine AS.

Recently we postulated that the importance of the ACA in primates was simply the result of the number of vessels (ACA N = 7 to 8, LPCA N = 2). If we assume that the two LPCA account for the majority of AS blood flow undisturbed by tenotomy in canines, there apparently is a greater perfusion per artery for the LPCA than for the ACA in dogs. However, it is impossible to determine whether the undisturbed AS flow represents the normal carrier volume of the LPCA, an increased "compensatory" volume resulting from stimuli associated with disruption of the ACA, or the contribution of both the LPCA and choroidal circulations to the AS.

The medial and lateral quadrants for the unit I,CB,CP had a higher blood flow than the superior and inferior quadrants in the control eye. Since the medial and lateral LPCA enter the outer aspect of the CB in the horizontal plane before dividing into branches, one might expect the horizontal quadrants to receive a slightly greater proportion of the blood carried by the LPCA than the vertical quadrants.

Hayreh and Scott noted persistent fluorescein filling delay in the corresponding iris segments after vertical recti tenotomy. They concluded that the superior and inferior ACA were the major suppliers of blood to the superior temporal and inferior temporal aspects of the iris whereas the medial LPCA supplies the nasal aspect in humans. If this were the case in canines, one should expect a substantial decrease in blood flow to the superior and inferior quadrants as well as a significantly reduced flow to the lateral as compared to the medial quadrant after complete tenotomy. However, we did not observe a selective reduction in blood flow to any one quadrant as compared to another in the experimental eye, indicating that the majority of blood flow to all four quadrants of the unit 1,CB,CP is not via the ACA in canines.

Since the canine is clearly unlike the primate in the distribution of ocular blood flow and the importance of the circulation via the ACA, the dog is an inappropriate animal model for studies dealing with the ischemic vulnerability of the AS after strabismus surgery or with the physiological relationship of the LPCA and ACA.

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REFERENCES