A light microscopic study of the effects of testicular hyaluronidase on the outflow system of a baboon (*Papio cynocephalus*)

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An attempt was made to investigate the effects of hyaluronidase on the morphology of the baboon outflow system. The eyes of six adult baboons provided the material for this histological study. For each animal the IOP in both eyes was maintained at 18 mm Hg, and 150 IU of testicular hyaluronidase were introduced into one eye and a control solution into the other. It was observed by light microscopy that the outer meshwork was more distended in the hyaluronidase-treated outflow system than in the corresponding controls. Further, it was found from a quantitative analysis that the incidence of giant vacuoles in the endothelium of Schlemm’s canal was greater in the experimental than the control eye of each animal. The difference between the vacuole counts in the experimental and control eyes was significant in five of the six animals. These preliminary findings provide morphological evidence which indicates that there is a hyaluronidase-sensitive barrier to aqueous outflow in the baboon drainage system.

Key words: baboon, outflow apparatus, Schlemm’s canal, giant vacuoles, testicular hyaluronidase

Recent ultrastructural studies using electron-dense cationic dyes have shown that carbohydrate-rich mucins are a component of the extracellular connective tissue in the primate outflow system. The precise nature of this material remains unknown, but it would appear to be a complex mixture of mucopolysaccharides, a proportion of which is sensitive to the rather nonspecific enzyme testicular hyaluronidase.

The pathways for the drainage of aqueous humour are at their narrowest and most tortuous immediately beneath the endothelium of Schlemm’s canal in the endothelial meshwork, and it is in this region that the heaviest concentrations of mucopolysaccharides occur. Thus it could be expected that the hydrophilic polysaccharide network strategically situated in the finest portion of the drainage system would make a significant contribution to trabecular resistance.

Attempts to demonstrate a hyaluronidase-sensitive barrier by facility measurements have provided variable results. An increase in the facility of aqueous outflow produced by hyaluronidase has been more convincing in nonprimates than in primates.

The present investigation was conducted in vivo on the eyes of baboons, and an attempt was made to assess the effects of hyaluronidase on the outflow tissues with the use of morphological criteria rather than facility measurements.

The giant endothelial vacuoles which
Table 1. IOP and giant vacuoles per section

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Status</th>
<th>Resting IOP (mm Hg)</th>
<th>Maintained IOP (mm Hg)</th>
<th>Giant vacuoles per section through Schlemm's canal (mean ± S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Trabecular wall</td>
</tr>
<tr>
<td>1</td>
<td>Treated</td>
<td>17</td>
<td>18</td>
<td>24.6 ± 7.4</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>17</td>
<td>18</td>
<td>15.9 ± 8.3†</td>
</tr>
<tr>
<td>2</td>
<td>Treated</td>
<td>15</td>
<td>18</td>
<td>27.4 ± 8.7</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>16</td>
<td>18</td>
<td>15.8 ± 8.8†</td>
</tr>
<tr>
<td>3</td>
<td>Treated</td>
<td>16</td>
<td>18</td>
<td>25.4 ± 9.2</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>16</td>
<td>18</td>
<td>21.5 ± 6.0</td>
</tr>
<tr>
<td>4</td>
<td>Treated</td>
<td>9</td>
<td>18</td>
<td>14.2 ± 5.4</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>8</td>
<td>18</td>
<td>12.9 ± 4.6</td>
</tr>
<tr>
<td>5</td>
<td>Treated</td>
<td>10</td>
<td>18</td>
<td>8.9 ± 3.1</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>12</td>
<td>18</td>
<td>6.3 ± 2.5*</td>
</tr>
<tr>
<td>6</td>
<td>Treated</td>
<td>10</td>
<td>18</td>
<td>19.9 ± 4.8</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>12</td>
<td>18</td>
<td>15.9 ± 7.4*</td>
</tr>
</tbody>
</table>

*p value control vs. treated *p < 0.05; †p < 0.001; ‡p < 0.001.

are found in the endothelial monolayer of Schlemm’s canal have been implicated in the process of aqueous transfer.7,13 Certainly their numbers are increased by elevation of the intraocular pressure (IOP),14-18 and high facility values and high outflow rates are associated with vacuolar abundance.19

The proposed hypothesis was that if hyaluronidase-sensitive mucopolysaccharides significantly hinder the passage of aqueous humour, then at constant IOP there should be more giant vacuoles in an outflow system perfused with active hyaluronidase than in one which was perfused with an inactive solution. This was tested by experiments in which both eyes of a baboon were maintained at the same level of IOP and in which hyaluronidase was introduced into one eye and a control solution into the other. The results obtained by light microscopy are presented in this report, and they include a quantitative assessment of the incidence of the giant vacuoles in the endothelium of Schlemm’s canal.

Materials and methods

The experiments were conducted on the eyes of six fully mature baboons (Papio cynocephalus) weighing between 11 and 15 kg. The animals were tranquillized with intramuscular phencyclidine (12 mg) and anesthetized with intravenous sodium thiopental (75 mg/kg) to a level at which the corneal reflex was absent. Therefore anesthesia was maintained with a gas mixture of 75% nitrous oxide and 25% oxygen via an endotracheal tube on an open system.

A femoral artery was cannulated, and mean arterial blood pressure was recorded throughout the experiments on a pressure transducer (Bell & Howell Co., Pasadena, Calif.). At half-hour intervals throughout the experiment femoral arterial blood samples were taken for the measurement of blood gases (PACO₂, PAO₂, and pH) so that hypoventilation or hyperventilation could be avoided.

Once a suitable level of anesthesia had been achieved, IOP was measured with a Perkins hand-held tonometer, and then a 23-gauge needle attached to a manometer system containing a mock aqueous solution20 was introduced into each eye. Thereafter IOP was maintained at 18 mm Hg in both eyes (Table I). From a second intracameral needle 0.1 ml of mock aqueous which contained 150 IU of testicular hyaluronidase (B.D.H. Chemicals, Ltd., Poole, England) was infused into the experimental eye. The infusion was carried out manually via a microsyringe attached to the needle by microbore polyethylene tubing. A volume of 10 μl was delivered every 3 min, so that the infusion took 30 min to complete.

In the other eye 0.1 ml of control solution was introduced into the anterior chamber in a comparable manner and over the same time period. With three baboons the control solution was Bárány’s mock aqueous, and in the other three baboons the mock aqueous contained 150 IU of testicular hyaluronidase deactivated by heat.

Subsequently both eyes were left undisturbed.
Fig. 1. Semithin sections which show part of the trabecular wall of Schlemm's canal in (A) a hyaluronidase-treated eye and (B) a control eye. It can be seen that the endothelial meshwork is more distended in the hyaluronidase-treated tissue than in the control. (x1000.)

at the maintained IOP for 2 hr. Thereafter a third needle which was connected to a reservoir containing 3% glutaraldehyde in Sorenson’s phosphate buffer was introduced into the anterior chamber. The reservoir containing fixative was adjusted to a height of 18 mm Hg, and the microtubing leading to the other two needles was clamped.

The buffered glutaraldehyde contained fluorescein dye as a marker, and direct observation of the anterior segment confirmed a rapid dispersion of fixative at this pressure level. Perfusion was allowed to continue for 30 min, during which time the animal was sacrificed by an overdose of pentobarbital sodium. The eyes were then enucleated and immersed in the primary fixative solution; the anterior segments were removed and cut into quadrants. Meridional samples of limbal tissue from each quadrant were washed in buffer, post-
fixed in 1% osmium tetroxide for 1 hr, and re-
washed in buffer solution. The tissue blocks were
then dehydrated through graded alcohols, cleared
with propylene oxide, and embedded in Araldite.
Semithin (1 to 2 µm) sections were cut on an LKB
Ultrotome III and stained with toluidine blue.

Results

There were no morphological differences
at the light microscopic level between the
outflow apparatus from the control eyes in-
fused with Bárány's solution and those in-
fused with deactivated testicular hyaluronidase.
The infusion of active testicular hyaluronidase into the anterior chamber of the
experimental eyes did not produce marked
changes in the trabeculae. There was no ob-
vious change in trabecular thickness, and the
endothelial cells remained adherent to the
trabecular cores. However, the outer mesh-
work was more distended in those eyes
treated with active hyaluronidase than in the
two sets of controls. The extracellular spaces
of the endothelial meshwork were more di-
lated and lucent than those in tissue which
had not been treated with active enzyme
(Fig. 1).

Giant vacuoles were present in the endo-
theelium of Schlemm's canal in both groups,
but it was our opinion that they were more
prevalent in the treated than the control
group. To test this impression in a more ob-
jective manner, a count was made of giant
vacuoles in semithin sections cut from coded
blocks. One section through Schlemm's canal
was examined from each of 16 blocks of tissue
(four blocks from each quadrant) for each eye
under investigation, and the counts were
made at ×1000 magnification with an oil-
immersion objective.

The results of this quantitative analysis
are shown in Table I. Giant vacuoles were
not found on the corneoscleral aspect of
Schlemm's canal; thus the column which rep-
resents giant vacuoles elsewhere in Schlemm's
canal are those which were present in
the endothelium on the septae bridging
Schlemm's canal and also those in the en-
dotheelium at the margins of the canal where
the trabecular and corneoscleral walls meet.

The number of giant vacuoles per section
was greater in the experimental than the con-
trol eye of each animal under investigation.
The difference obtained from the counts of
total giant vacuoles per section was sig-
nificant in all but one animal (no. 4). How-
ever, the percentage increase in incidence
was inconsistent and ranged from 13.4% to
78.4%. The over-all average increase in vac-
ular incidence in the six experimental eyes
was 48.1%, but interestingly, the increase in
vacular incidence was greater in the three
animals in which Bárány's solution served as
the control infusion fluid (60.9%) than in the
other three animals where the infusate into
the control eyes was deactivated enzyme so-
lution (35.3%).

Discussion

It has been shown that the infusion of tes-
ticular hyaluronidase into the anterior cham-
ber of the baboon eye produced observable
differences in the appearance of the outflow
system when compared to control eyes. In
particular, distention of the endothelial meshwork and an increase in the incidence of
giant vacuoles in the endothelium of
Schlemm's canal were noted.

If it is accepted that vacuolar abundance is
associated with situations where the outflow
rate is high,13-19 it could be argued that per-
fusion with hyaluronidase increased conduc-
tance through the trabecular meshwork by
depolymerization of the hydrophilic carbo-
hydrate-rich mucins. Hence, at constant
IOP, the flow rate to the canal endothelium
would be increased, leading to greater num-
bers of giant vacuoles in the canal endo-
dotheelium.

The possibility remains that either (1) flow
rates were not increased by perfusion of hy-
aluromidase (and we have no physiological
measurements to prove that they were) or
(2) the giant vacuoles may be degenerative
structures produced by the toxic effects of the
enzyme on the endothelial cells.

If rates of aqueous outflow and facility val-
ues had been measured, these data would
have provided the information with which
the hydrodynamics of aqueous outflow and
vacuolar incidence could be correlated. It was, however, valid to keep the initial experiments as simple as possible because it has been shown that the manipulations involved in manometric facility determinations can cause disruptive changes to the delicate meshwork tissues which may have prejudiced the findings.\(^{19}\)

Shabo et al.\(^{21}\) have considered that giant vacuoles are degenerative structures produced by postmortem changes or inadequate fixation. This conclusion has been disputed by various other groups, who have concluded that the vacuoles are "real" entities.\(^ {13, 15-19}\)

From preliminary electron microscopic studies on the present tissue we have found that the giant vacuoles described in this light microscopic study have the characteristics of the pressure-sensitive vacuoles which have been implicated in the transendothelial transfer of aqueous humor.\(^ {16, 18, 19}\)

In the light of the inconsistency in previous perfusion studies with hyaluronidase and the lack of data on changes in flow dynamics in the present investigation, there is need for further research. A combined ultrastructural and physiological study of hyaluronidase effects on the drainage tissues would be a reasonable extension of this initial report.

REFERENCES