Effect of intravitreal hyaluronidase on the clearance of tritiated water from the vitreous to the choroid

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SUMMARY   The rate of transfer of intravitreally injected tritiated water from the mid vitreous to the
choroid is significantly increased after depolymerisation of vitreous hyaluronic acid by injected
hyaluronidase. The significance of this finding is discussed in relation to such conditions as retinal
detachment and reattachment.

We have previously described a method for studying
the transfer of intravitreally injected tracers from the
vitreous to the choroid. The major proportion of
intravitreally injected tritiated water is rapidly
cleared to the choroid across the cortical vitreous,
retina, and retinal pigment epithelium (RPE).[1] This
movement of water from the vitreous to the choroid
may be of importance in the maintenance of retinal
apposition.[2][3] We were interested to see whether
modification of the physical characteristics of the
cortical vitreous gel by depolymerisation of its con-
tained hyaluronic acid would alter the rate of transfer
of tritiated water from the mid vitreous to the
choroid.

Hyaluronic acid in the vitreous is an important
structural component,[4] and, although this highly
polymerised hydrophilic molecule is likely to inter-
fere with bulk movement of water, it has been
suggested that diffusional water movement would
not be so influenced (Balazs E A, personal communi-
cation). In the present series of experiments we have
measured the clearance of intravitreally injected
^3H_2O in rabbit eyes in which the vitreous hyaluronic
acid had been deploymerised by previously injected
hyaluronidase and have compared the results with
earlier measurements on normal rabbit eyes.[1]

Materials and methods

The experiments were performed on a group of six
adult male Dutch rabbits weighing between 1·6 and
2·4 kg and compared with control experiments
carried out on a group of nine animals of similar
strain, age, and weight. The animals were anaesthe-
tised with intravenous ethyl carbamate (Urethane) in
a dose of 1·9 mg/kg body weight. Subsequently 750
international units of bovine testicular hyaluronidase
(Hyalase; Fisons Ltd, Pharamaceutical Division) in
0·1 ml water was injected via the pars plana ciliaris
into the middle of the vitreous cavity of one eye with a
microsyringe and a 26 gauge needle. The injecting
needle was kept in' situ during the rest of the
experiment.

Approximately 30 minutes after the injection of
hyaluronidase 25 µCi of ^3H_2O in 25 µl of water was
injected into the mid vitreous through a separate
injection site in the pars plana ciliaris, and this needle
too was kept in situ while clearance of the injected
^3H_2O to the choroid was measured by the technique
previously described.[1] In summary, the upper nasal
vortex vein was identified, cleared of surrounding
tissue, and divided. Blood was collected from its cut
end into long form Pasteur pipettes under the
influence of capillarity and gravity, the pipettes being
changed at two-minute intervals or more quickly if
the blood flow was great enough to justify it. Blood
samples were weighed, decolourised, and mixed with
scintillation fluid (NE 260, Nuclear Enterprises Ltd),
and the activity of ^3H in the blood samples was
assayed by liquid scintillation counting. At the end of
the experiment the animal was killed and the eye
enucleated. The number of vortex veins was counted,
and the state of the vitreous was determined by
examination of the bisected globe with an operating
microscope.

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In control experiments the experimental method was similar to that used in the hyaluronidase experiments, except that the injection of intravitreal hyaluronidase was omitted. The results of control experiments in normal eyes have been previously reported.[1] In relation to the state of the vitreous as examined under the operating miscroscope, the fellow eye in animals in which intravitreal hyaluronidase had been injected in one eye was used as a control.

Results

As had previously been found in control experiments,[1] all eyes had four vortex veins. There was, however, a marked difference in the state of the vitreous in eyes receiving hyaluronidase as compared with normal rabbit eyes, the vitreous in the former being liquid and rapidly running out of the bisected globe, unlike the latter in which the vitreous retained its firm gel-like characteristics.

In our initial study of normal rabbits the parameter used to characterise the rate of appearance of the ^3H_2O in choroidal blood was the mean transit time, the average time taken for a molecule of ^3H_2O to be cleared from the eye. However, the value of this quantity depends on the duration of the experiment, and the use of this parameter is inappropriate where experiments of differing lengths must be compared. In the present study control experiments lasted 80 minutes and those on rabbits injected with hyaluronidase only 60 minutes.

The difficulty in making comparisons between these two groups of experiments was resolved by fitting an exponential decay to the decrease in ^3H concentration in the blood samples after the maximum value had been reached several minutes after the start of the experiment. Examples of fitted decays
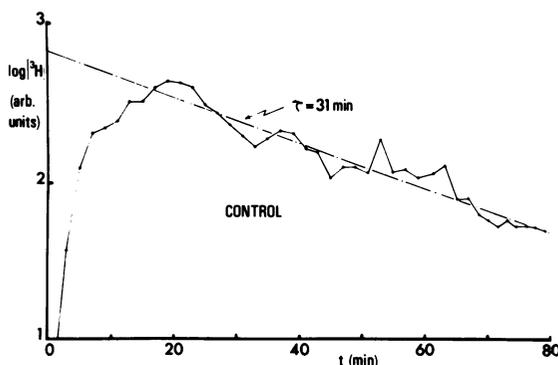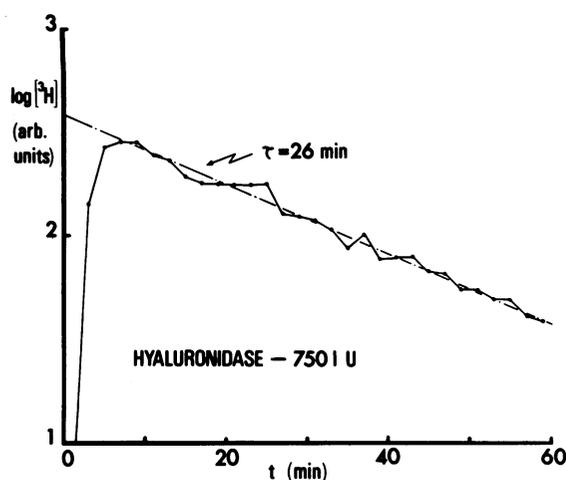

Fig. 2 *Application of an exponential decay to the decline in ^3H concentration in blood samples from a vortex vein after an intravitreal injection of ^3H_2O in an eye in which the vitreous hyaluronic acid had been depolymerised by the injection of hyaluronidase.*

are shown in Figs. 1 and 2 by the dot-dash lines and it is evident that these do give a good description of the variation of the data over much of the experimental time. We therefore took the time constant, τ, for the fitted decay as the index of rapidity of clearance of ^3H. τ has no intrinsic dependence on the length of experiment; smaller values of τ correspond to a more rapid removal of tritiated water.

The values of τ obtained from the experiments on the rabbits treated with hyaluronidase are detailed in Table 1 together with values obtained from reanalysing the control experiments described previously.[1]

Table 1 *Rate of movement of ^3H_2O from vitreous to choroid in normal rabbit eyes and in eyes injected with intravitreal hyaluronidase. The rate of clearance of ^3H_2O from vitreous to choroid is increased in hyaluronidase treated eyes*

Time constant (min)	
Control eyes	Hyaluronidase treated eyes
54	23
24	12
25	28
26	26
30	13
31	18
47	
33	
56	
Mean 36	20
SD 13	7
SE 4	3

$0.01 < P < 0.02$ (Mann-Whitney test).

Fig. 1 *Example of exponential decay fitted to rate of decline of ^3H concentration in vortex vein blood samples after intravitreal injection of ^3H_2O in a normal rabbit eye.*

Table 2 *Total recovery of ^3H_2O from one vortex vein after intravitreal injection of ^3H_2O in normal eyes and in eyes receiving an intravitreal injection of hyaluronidase*

Amount of injectate recovered (per cent)

Control eyes	Hyaluronidase treated eyes
18	30
38	54
23	14
35	14
27	13
24	12
26	
24	
13	
Mean 25	23
SD 8	17
SE 3	7

$0.2 < P < 0.5$ (Mann-Whitney test).

The mean time constant for the hyaluronidase series was 20 ± 3 minutes (mean $\pm$ standard error) compared with 36 ± 4 minutes for the control group. The difference between the two sets of time constants was tested by the non-parametric Mann-Whitney test and found to be significant at the 5% level ($0.01 < p < 0.02$; two-tailed).

The total recoveries of ^3H_2O are set out in Table 2 with the control data for comparison. A Mann-Whitney test showed no significant difference between the two groups of data, though the trend was for lower recoveries in the hyaluronidase series perhaps because of the shorter durations of experiments in this group.

Discussion

Although the results of the experiments on water movement from the vitreous to the choroid in normal control animals fitted the pattern expected for diffusional movement of water from vitreous to the choroid, we have not excluded the possibility of a pressure-dependent component of flow or indeed a movement dependent upon active transport in the pigment epithelium or resulting from the colloid osmotic pressure difference between the vitreous and the protein-rich extravascular spaces of the choroid.

The control animals used in these experiments were of the same strain, weight, and age as the animals receiving intravitreal hyaluronidase, and the experiment itself was similar in both groups of animals with the exception of the introduction of hyaluronidase into the eyes of the experimental group. In some respects it might have been better to have used the fellow eye of the same animal as a control, but this was precluded by the impossibility of achieving adequate access to vortex veins in each eye simultaneously. The question of leakage round the injection sites of either the tritiated water or the hyaluronidase had also to be considered. Experiments in which fluorescein was mixed with the injectate showed no evidence of leakage round the injection sites when the technique described was used, nor were significant differences found between the recovered amounts of injectate in control and experimental eyes (Table 2), which would have been expected had there been significant loss of injectate round the injection site in eyes receiving intravitreal hyaluronidase.

From the data presented here it does appear that vitreous hyaluronic acid can influence the diffusional movement of water in the vitreous. In the normal eye the polymerised hyaluronic acid is evidently a rate limiting factor, possibly one of several, in the processes of water transport from mid vitreous to choroid, since the rate of water transfer has been shown to be faster in eyes treated with hyaluronidase, and it is known that hyaluronidase depolymerises vitreous hyaluronic acid.[5 6]

It has long been accepted that the vitreous structure could act as a barrier to the passage through it of large protein molecules, cells, and the like, and indeed it has been shown that hyaluronic acid in various concentrations systematically reduces the diffusion of chemotactic peptides in solution[7] and also the movement of macrophages.[8] The question of the influence of hyaluronic acid on water movement has been less certain. Ogston and Sherman[9] showed that solutions of hyaluronic acid stabilised on micropore filters could slow down water flow, but Balazs (personal communication) has refuted the suggestion that hyaluronic acid in the vitreous would influence diffusional movement of water. On clinical grounds it has been suggested that intact vitreous gel may be capable of blocking open retinal holes, in some cases preventing retinal detachment.[10] It has also been postulated that vitreous tamponade of retinal holes in volume-reducing operations for retinal detachment may be important in relation to surgical cure of such detachments.[10] In particular it has been suggested that vitreous tamponade of a retinal hole might explain the flattening of the retina which commonly occurs after episcleral plombage where the retinal hole is not in contact with the indentation in the immediate postoperative period.[10]

The present study lends support to the view that intact cortical vitreous gel can limit the movement of water molecules, confirming that vitreous tamponade of retinal holes is a physical possibility.

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