Reports

Retinal Adhesiveness in the Monkey
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Purpose. To determine factors that influence retinal adhesion in the primate and compare these with previous data from the rabbit.

Methods. Retinal adhesiveness was studied in monkey eyes immediately after enucleation. The retina was peeled manually from the retinal pigment epithelium, and the amount of pigment that remained adherent to the retina was used as an index of adhesiveness.

Results. The rate of post-mortem failure of retinal adhesiveness in the monkey was less than in the rabbit under similar conditions. However, as in the rabbit, adhesiveness was sensitive to temperature, pH, and the concentrations of calcium and magnesium, and subretinal injections of neuraminidase weakened adhesion beyond the injection sites.


Much of the available information on factors that influence retinal adhesion has been gathered from the rabbit. Retinal adhesiveness in this species, as measured by peeling the retina from retinal pigment epithelium (RPE) in excised tissue, falls rapidly within the first few minutes after enucleation, but can be maintained for hours at low temperatures. Retinal adhesiveness is weakened reversibly by either low pH or a low-calcium, low-magnesium environment. Retinal adhesiveness can also be weakened by exposing components of the interphotoreceptor matrix (IPM) to neuraminidase or chondroitinase ABC. Kita et al have measured retinal adhesion in the living rabbit eye, and found a similar dependence of retinal adhesive force upon calcium.

The relevance of these data to humans has been unclear because in vitro experiments might not reflect the true balance of retinal adhesive factors in the living eye, and because adhesive mechanisms in primate eyes may differ from rabbit eyes. This report presents the results of experiments on retinal adhesive factors in excised primate tissue for comparison with rabbit data obtained under similar conditions.

METHODS. All experiments conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Stanford Administrative Panel on Laboratory Animal Care. The effects of pH; calcium and magnesium; temperature; and time after enucleation were studied on eyes obtained from eight adult cynomolgus monkeys (estimated to be 5 to 7 years old). The experiment on enzyme effects was performed on one adult Japanese monkey. Cynomolgus monkeys were anesthetized with intramuscular ketamine, 10 mg/kg and pentobarbital sodium, 24 mg/kg. Pentobarbital was added (5 mg/kg) as needed during the experiment. The Japanese monkey was anesthetized with intramuscular thiamylal sodium, 25 mg/kg and ketamine hydrochloride, 15 mg/kg. The eyes were enucleated before euthanasia, except for the eye used for the study on the reversibility of temperature effects, which was enucleated within 2.5 minutes of death.

The following reagents and solutions were used: neuraminidase (NA), type X, from Clostridium perfringens (type X is a further purification by affinity chromatography of NA type VIII; Sigma, St. Louis, MO); Hanks’ solution (Gibco, Grand Island, NY); disodium ethylenediaminetetraacetic acid (Sigma); Hanks’ solution without calcium and magnesium, plus 1 mM disodium ethylenediaminetetraacetic acid (Sigma); and Ames’ solution (Sigma).

For subretinal injection of enzyme, a glass micropipette with tip diameter of 40 to 50 μm was passed through a scleral slit near the limbus and across the vitreous until the tip just penetrated the central retina. Air pressure forced Hanks’ solution with or without neuraminidase (1.7 U/ml) into the subretinal space, creating a 3- to 3.5-mm-diameter retinal detachment.

The degree of adherence of RPE pigment to the neural retina after peeling from the RPE in vitro was used as a quantitative index of adhesiveness. In brief, enucleated eyecups were rapidly cut into strips. For the enzyme study, the strips included the areas of experimental detachment. The retina was peeled manually from the RPE while submersed in an experimen-
tal bath of Hanks' solution or Ames' solution, modified according to the experiment. The solutions were held in a Petri dish and were not circulated or oxygenated during the experiment. The strength of retinal adhesion was estimated by measuring the area of retina that was covered by adherent RPE pigment (100% adherent pigment indicated firm adhesion; 0% adherent pigment indicated weak adhesion).

Direct-current electroretinograms were recorded between the cornea and a reference point on the sclera, using Ag–AgCl electrodes. For scanning electron microscopy, pieces of peeled neural retina or RPE-choroid-sclera were pinned to sheets of wax, and prepared as described previously. Histocytochemistry with the fluorescein-conjugated lectins peanut agglutinin (PNA) and wheat germ agglutinin (WGA) was performed according to our established method.

RESULTS. Postenucleation Failure of Adhesion. Figure 1 shows the changes in pigment adherence relative to time after enucleation, for primate tissue kept in Hanks' or Ames' solution at 36°C to 37°C. During the first 10 minutes after enucleation, the peeled primate retina was 90% to 100% covered with adherent pigment. Thereafter the percentage dropped, and reached low levels by 20 to 40 minutes after enucleation. The more rapid time course of postenucleation adhesive failure in the rabbit is shown for comparison. The interpretation of 0% pigment adherence as weak adhesiveness could be erroneous if outer segments were fragmenting instead of RPE microvilli. However, scanning electron microscopy of the RPE surface after pigment adhesion had fallen near 0% showed no adherent outer segment fragments.

Effect of pH and Calcium. Figure 2 shows the results from two eyes in which paired strips of eyecup were incubated in Hanks' solution at 36°C to 37°C in normal (pH 7.4) and pH 6.0 solutions, and peeled at the same time. Reducing the pH accelerated the rate of retinal adhesive failure after the initial 5 minutes. Previous results from the rabbit are shown for comparison. Figure 3 shows a similar experiment in which the paired strips were incubated in normal Hanks' solution or in Hanks' solution without calcium or magnesium. The removal of calcium and magnesium accelerated the rate of retinal adhesive failure after the initial 5 minutes of incubation. Previous data from the rabbit are again shown for comparison.

Effect of Temperature. Adhesiveness diminished at a much slower rate at room temperature (16°C to 20°C) than at 36°C to 37°C (Fig. 4). At room temperature, peeled retinas showed 100% pigment adherence until roughly 35 minutes after enucleation, and the
percentage did not fall until nearly 1 hour after enucleation. We also studied one eye in which the tissues were initially placed into Hanks' solution at 37°C, and pigment adherence followed until it fell to 0% at 26 minutes after death; transfer of unpeeled segments to a bath at 4°C caused partial recovery of pigment adherence to 30% after 15 minutes.

**IPM and Enzyme Effects** Some aspects of IPM structure are revealed by staining with fluorescein-conjugated lectins. PNA binds to the cone matrix sheaths, whereas WGA binds to rod outer segments and associated matrix material. We used these cytochemical markers to see whether histologic changes in the IPM could be correlated with retinal adhesiveness. Figure 5 compares IPM morphology immediately after enucleation when pigment adherence was 100% with that after 18 minutes incubation at 37°C when adherence was 10%. The PNA staining (cone sheaths) shows little difference, but the 18-minute WGA tissue shows some disruption of rod outer segments.

We investigated, in one eye, the effects of enzymatic disruption of the IPM. Neuraminidase solution was injected into the subretinal space, and two days later the eye was enucleated to measure adhesion. In rabbits, this time was sufficient for enzyme to diffuse laterally and weaken adhesion far beyond the injection site. The primate eye injected with neuraminidase showed an area of weakened adhesiveness (low pigment adherence) extending 2 to 3 mm beyond the site of the enzyme injection. Scanning electron microscopy showed neither photoreceptor nor RPE damage in this area. Electroretinograms were recorded from the neuraminidase-injected eye before and 2 days after the injection of the enzyme, to rule out widespread retinal damage. There were no changes in the a-, b-, or c-waves. Sections were cut from this eye to correlate the degree of adhesiveness with PNA binding to the IPM. We found (Fig. 6) abnormal diffuse PNA binding, characteristic of neuraminidase damage in the region of weakened pigment adherence, whereas further away from the injection site (where pigment adherence was a normal 100%) we found a normal pattern of PNA binding.

To document more directly the role of matrix in connecting retina to RPE, we peeled some tissue samples only partially so that the degree of matrix traction at the point of separation could be examined. Staining for PNA and WGA binding showed that 2.5 minutes after enucleation, there was slight stretching (approximately 1.2 to 1.4 times that of normal length) of both cone and rod matrix across the subretinal space. By 4

**FIGURE 4.** Change in retinal adhesiveness with time after enucleation at reduced temperature. Tissues from four eyes (two monkeys) incubated in Hanks' solution at 16°C to 20°C. Rabbit data at 16°C to 20°C are shown for comparison.

**FIGURE 5.** Immunofluorescent light micrographs of retinas stained with either PNA to delineate cone matrix sheaths (A, B) or WGA to delineate rod photoreceptor outer segments and associated IPM (C, D). Within each pair, the first was peeled immediately after enucleation and showed 100% pigment adherence to peeled neural retina (A, C), and the second was peeled after 18 minutes of incubation in 37°C Hanks' solution and showed only 10% pigment adherence (B, D). The distribution and morphology of IPM domains appeared normal at both time points; however, disruption or disorganization of rod outer segments was apparent with the WGA stain at 18 minutes (D).
was no longer observed.

minutes or longer after enucleation, this stretching was no longer observed.

DISCUSSION. The results show that retinal adhesion in the monkey, as measured by peeling the retina from strips of excised eyecup, diminishes with time after enucleation, is sensitive to temperature, pH and calcium, and can be weakened by neuraminidase. These characteristics are qualitatively similar to findings in the rabbit obtained by this technique of measurement, and suggest that similar mechanisms of retinal adhesion exist among mammalian species. One implication is that agents that may be shown to facilitate adhesion in the rabbit are likely to have similar effect in the primate and may be of potential clinical value in humans.

We recognize that the in vitro measurement of adhesiveness in terms of pigment adherence to peeled retina is an indirect measure and is subject to post mortem changes and other sources of error. For example, if photoreceptor outer segments were damaged by an experimental condition, retinal separation might occur through breakage of the outer segments while adhesion to RPE remained strong. However, we found little or no outer segments adherent to peeled RPE by scanning electron microscopy. In vivo measurement of adhesion would have been preferable, but the technique only allows measurement of a single condition within an eye, and experimental variability is such that we could not have analyzed all of our conditions with the small number of primates available to us. The peeling technique allows measurements within about 1 minute of enucleation, and past experiments have shown good correlation in physiologic effects between different methods of adhesion measurement. We have tried to minimize the inherent variability of individual pigment adherence measurements in these experiments by studying paired sets of primate tissue from the same eye whenever possible. Thus, even though only two eyes were used for the calcium and pH experiments, we found consistent effects in corresponding regions of the same eye handled identically except for differences in ionic concentration. Because the results also agree with earlier rabbit data, we expect that they apply to primates in general.

The disappearance of a strong retinal adhesive bond with a few minutes of enucleation suggests that adhesive mechanisms require metabolic support, most likely metabolic ion and fluid transport across the RPE. RPE transport controls the relative dehydration of the IPM, and the ionic balance within it, which may well be critical for firm receptor-ligand complexes. It is of interest that IPM morphology correlated with retinal adhesive strength with respect to enzyme-induced (neuraminidase) damage, but not with respect to time after enucleation. As adhesion fell during post mortem incubation at 37°C, there was some visible photoreceptor disorganization but no obvious disruption of the WGA- or PNA-stained IPM material. This suggests that IPM digestion or dissolution is not the initial cause of post mortem adhesive failure, but that there must be a loss of the binding effectiveness of the IPM, perhaps for reasons of metabolic failure noted above.

Although we have emphasized similarities, there are also differences in retinal adhesiveness between primate and rabbit. For example, retinal adhesiveness is more resistant to post mortem degradation in the primate, and recent in vivo measurements of adhesive force have shown the force of adhesion to be 1.4 times greater in primate than in rabbit. Some of these differences may relate to the fact that primate IPM (and especially the cone matrix sheaths) has a much greater volume than in rabbits.

We presume that primate eyes are physiologically closer to human eyes than rabbit eyes with respect to retinal adhesion, especially because the RPE and outer retina are more alike (for example, rabbits have few cones and a thinner RPE). This assumption is not necessarily valid for all physiologic processes (for example, the vitreous varies in consistency among primate species), but some surgically enucleated human eyes have now been studied with comparable techniques and the mechanisms of adhesion appear to be qualitatively similar to those in primates.

Key Words
interphotoreceptor matrix, primates, retinal adhesion, retinal pigment epithelium
Purpose. Pseudoexfoliation (PSX) eyes frequently show clinical signs of blood–aqueous barrier impairment. To analyze these alterations, the authors examined aqueous humor of human eyes with and without PSX.

Methods. After aqueous humor samples had been obtained during cataract or filtering glaucoma surgery, a modified Pierce-bicinchoninic acid assay was used to quantify total aqueous protein concentration in 27 PSX eyes and 59 eyes without clinical signs of PSX (12 cataract eyes and 25 eyes with primary open-angle glaucoma). Aqueous humor was obtained during intraocular surgery from 27 eyes of 27 patients (age 72.6 ±9.1 years; 16 men and 11 women) without any clinical signs of PSX with various clinical diagnoses. All patients underwent a complete ophthalmologic examination, and the type and severity of cataract and the presence and morphologic revealed a characteristic increase of a 12.5-kDa band in 15 of 27 PSX eyes but in only 1 of 59 eyes without PSX (P < 0.00001, chi-square test).

Results. Aqueous protein concentration was significantly higher in PSX (mean 0.42 ± 0.16 mg/ml) than in normal cataract eyes (0.22 ± 0.08 mg/ml, P < 0.0001) and in eyes with open-angle glaucoma (0.26 ± 0.09 mg/ml, P < 0.0001, Wilcoxon-Mann-Whitney test). Electrophoresis revealed a characteristic increase of a 12.5-kDa band in 15 of 27 PSX eyes but in only 1 of 59 eyes without PSX (P < 0.00001, chi-square test).

Conclusions. These results substantiate increased aqueous protein concentration and aqueous barrier impairment in PSX. The additional finding of an increased 12.5-kDa band in 56% of PSX eyes may be related to the pathogenesis of PSX in the anterior ocular segment. Investig Ophthalmol Vis Sci. 1994;35:748–752.

Biomicroscopic examination of pseudoexfoliation (PSX) eyes frequently reveals signs of discrete intraocular inflammation such as aqueous flare and postoperative fibrin formation. These findings appear to be related to alterations of the blood–aqueous barrier in PSX that have been observed using various methods such as iris fluorescein angiography,1 and fluorophotometry.1 In addition, quantitative assessment of aqueous flare with the laser flare-cell meter revealed significantly increased flare values in PSX eyes indicating increased aqueous protein concentration.2

It was the aim of this study to search for alterations of the aqueous humor in PSX eyes. Therefore, we performed both quantitative determination of total aqueous protein and qualitative assessment of the protein composition in eyes with and without PSX.

METHODS. Materials. Aqueous humor was obtained during intraocular surgery from 27 eyes of 27 patients (age 72.6 ± 9.1 years; 16 men and 11 women) with the characteristic clinical signs of PSX and from 72 eyes of 72 patients (age 53.9 ± 21.9 years; 33 men and 26 women) without any clinical signs of PSX with various clinical diagnoses. All patients underwent a complete ophthalmologic examination, and the type and severity of cataract and the presence and morphol...