Retinal Adhesive Force in Living Rabbit, Cat, and Monkey Eyes

Normative Data and Enhancement by Mannitol and Acetazolamide

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Small retinal detachments (blebs) were made in living eyes by injecting balanced salt solution into the subretinal space with a micropipette. A second micropipette, inserted into the same bleb, measured subretinal pressure using a resistance servonulling system. The adhesive force was calculated from the pressure difference across the retina according to Laplace's law. The retinal adhesive force in rabbit, cat, and monkey eyes averaged 1.0, 1.8, and $1.4 \times 10^2$ dyne/cm, respectively. In rabbit eyes, 2 hr after intravenous administration of 15 mg/kg acetazolamide, the retinal adhesive force was increased to 133%. In monkeys, this dose of acetazolamide increased retinal adhesion to 144% of control values. Mannitol (2 g/kg) increased retinal adhesion in the monkey to 153% of control values 90 min after intravenous injection (compared with an increase of 145% in previous experiments in the rabbit). Because both mannitol and acetazolamide enhance retinal adhesiveness in living primate eyes, it seems likely that they will have a similar effect in humans that they may be clinically useful.

Retinal detachment occurs when destructive factors, such as traction forces, overcome the constructive factors that normally maintain retinal adhesion. Modern surgical techniques allow us to repair most rhegmatogenous retinal detachments mechanically but at considerable cost and with varying degrees of success. Recent studies show that normal retinal adhesion is a multifactorial process, and this might allow us to apply various therapeutic strategies. However, pharmacologic management of retinal detachment has not been seriously considered to our knowledge, in part because we still lack information about the retinal adhesive forces in vivo and about pharmacologic effects on adhesion in primate eyes.

Most of the recent studies of retinal adhesion have been done with rabbit eyes, which generally are believed to have weaker retinal adhesion than eyes of higher species. In this study, we found species differences in retinal adhesive force as measured in living eyes of rabbits, cats, and monkeys, and we investigated the degree to which systemic mannitol and acetazolamide could enhance the retinal adhesive force.

Materials and Methods

These investigations adhered to the ARVO Resolution on the Use of Animals in Research. Dutch rabbits weighing approximately 1.5 kg were sedated and anesthetized with intramuscular injection of xylazine hydrochloride (2 mg/kg), acepromazine maleate (1 mg/kg), and ketamine hydrochloride (20 mg/kg). Cats weighing 3–4.6 kg were sedated with intramuscular ketamine hydrochloride (10 mg/kg) and anesthetized with intravenous pentobarbital sodium (5 mg/kg). Rhesus monkeys weighing 3.8–7.9 kg were sedated with intramuscular ketamine hydrochloride (10 mg/kg) and anesthetized with intravenous pentobarbital sodium (25 mg/kg). Their pupils were dilated with phenylephrine hydrochloride 10% and atropine sulfate 1% drops.

The retinal adhesive force was measured using our in vivo method. In brief, a small dome-shaped retinal detachment (bleb) was made by injecting Hanks balanced salt solution (Gibco, Grand Island, NY) through a glass micropipette into the subretinal space, using gentle air pressure. A second micropipette with a tip diameter of 5 μm was inserted into the same bleb and connected to a micropressure measuring system (World Precision Instruments, Grand Island, NY). More solution was injected through the first pipette to
elevate the subretinal pressure and enlarge the bleb. The subretinal pressure ($P_s$) was measured using the resistance servonulling method. The intravitreal pressure ($P_v$) was kept at a constantly low level by the wide scleral slits and measured before and after determining $P_v$. The retinal adhesive force ($A$) was defined as the force needed to achieve adhesive failure per unit length of the bleb margin. At the moment that the bleb just begins to expand, all forces exerted on the bleb must be balanced. $A$ was calculated from $P_s$, $P_v$, and the radius of the bleb ($r$) at the moment using Laplace's law ($P_s - P_v = 2A/r$).

We used mannitol 25% (Abbott, North Chicago, IL) and acetazolamide sodium (Quad, Indianapolis, IN) dissolved in distilled water (50 mg/ml) and administered intravenously after measuring a control value of the adhesive force.

After measuring adhesion, some eyes were enucleated for scanning electron microscopic study to confirm that separation was occurring in the subretinal space. The tissue was pinned to a sheet of wax and fixed with glutaraldehyde 1.25% and paraformaldehyde 1.0% in 0.072 mol/l sodium cacodylate buffer, pH 7.4. The tissue was dehydrated with ethyl alcohol and critical-point dried before routine coating and examination with a scanning electron microscope.

**Results**

We observed casually over the years that experimental detachments in monkeys and cats tend to form higher domes than in the rabbit and that they seem to fill and spread more slowly at the fluid pressures that we used. This suggested to us that the adhesion of the retina to the retinal pigment epithelium might be stronger in monkeys and cats. Table 1 shows the results of direct in vivo measurements of the retinal adhesive force in these species. The retinal adhesive force in cat eyes (tapetal area) and monkey eyes (posterior pole) was 180% and 140% of that in rabbit eyes, respectively.

Figures 1 and 2 show the time course of change in retinal adhesive force in rabbits after intravenous injections of 15 and 50 mg/kg of acetazolamide, respectively. Each symbol indicates measurements from one rabbit in relation to the control value (100%) from one eye of that rabbit, and measurements in the same eye are connected by dotted lines. After administering 15 mg/kg of acetazolamide (a dose comparable to that used in humans for lowering intraocular pressure), the retinal adhesive force increased to 133% of control values after 120 min, and then returned to normal by 300 min. The higher dose of 50 mg/kg showed a slightly stronger and more persistent effect.

Figure 3 shows the time course of changes in retinal adhesive force after administration of 15 mg/kg of acetazolamide in the monkey. These results were similar to those in the rabbit, with a maximum increase in adhesive force averaging 144% of control values at 150 min after injection.

<table>
<thead>
<tr>
<th>Species</th>
<th>$n$</th>
<th>Retinal adhesive force ($\times 10^2$ dyn/cm)*</th>
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</thead>
<tbody>
<tr>
<td>Rabbit</td>
<td>25</td>
<td>1.0 ± 0.07</td>
</tr>
<tr>
<td>Cat</td>
<td>4</td>
<td>1.8 ± 0.18</td>
</tr>
<tr>
<td>Monkey</td>
<td>6</td>
<td>1.4 ± 0.03</td>
</tr>
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</table>

* Mean ± SE.
We previously showed that mannitol (2.5 g/kg) increases retinal adhesiveness by 145% in the living rabbit eye (30–70 min after injection). Figure 4 shows the results of giving 2 g/kg of mannitol to monkeys. The retinal adhesive force was increased maximally 90 min after mannitol administration to an average of 153% of control, and it returned to normal 150 min after injection.

Scanning electron microscopy of selected eyes showed that the surface structure of both photoreceptors and retinal pigment epithelium was preserved with minimum damage during the formation of these bleb detachments in all three species.

Discussion

Although there is an anecdotal awareness that retinal adhesion seems stronger in primates or cats than rabbits (eg, in one study, cat retina seemed to have greater adhesiveness than rabbit retina, judging by the difficulty of starting bleb detachments), we are unaware of any previous systematic study of this issue. Our results show that the strength of retinal adhesion in living primates and cats is greater than that in the rabbit.

From a clinical standpoint, there are many reasons to seek drugs that would enhance retinal adhesiveness although there is no medical therapy available for retinal detachment currently. In vitro experiments have suggested that both mannitol and acetazolamide might be effective. We subsequently reported that systemic mannitol enhanced retinal adhesion in living rabbit eyes to 145% of control, and this effect disappeared approximately 90 min after administration.

In the current study, we found that acetazolamide also will enhance retinal adhesion in living rabbit eyes and, furthermore, that both mannitol and acetazolamide are effective in primates. The effect of mannitol in monkeys was slightly stronger, slower, and more prolonged than in the rabbit. The effect of a clinical dose of acetazolamide was slightly stronger in monkeys than in rabbits, but the time course of the effect was similar in the two species.

The mechanisms by which these agents increase adhesion are still not proved. Mannitol and acetazolamide both have been reported to hasten subretinal fluid absorption, the former by increasing osmotic flow out of the subretinal space and the latter by enhancing fluid transport across the retinal pigment epithelium. Dehydration of the subretinal space would be followed by an increase in viscosity of interphotoreceptor matrix, by possible changes in the behavior of glycosaminoglycans in the interphotoreceptor matrix that bridge the subretinal space, and by tightening the interdigitation between the photoreceptor and retinal pigment epithelial cells.

Because clinical doses of mannitol and acetazolamide increase retinal adhesiveness in living primates, these agents would seem to be promising clinically to prevent detachment, minimize the rate of enlargement of detachments, enhance the speed of reattachment, or reduce the risk of redetachment. We recognize that both drug effects are of limited magnitude and duration. It remains to be shown whether repeated doses can maintain a state of enhanced adhesion and whether these effects are large enough to alter the course of human disease. Local, rather than systemic, administration of acetazolamide might pro-
vide a means of safely delivering higher doses for more prolonged periods.

Key words: retinal adhesive force, retinal detachment, monkey, cat, rabbit, mannitol, acetazolamide

References