Topical vanadate lowers intraocular pressure in rabbits

Theodore Krupin, Bernard Becker, and Steven M. Podos*

In unanesthetized rabbits the topical application of vanadate lowered intraocular pressure. Tonographic outflow facility and episcleral venous pressure were unaltered by topical vanadate. As estimated from the tonographic data, aqueous humor flow was reduced by approximately 30%. Posterior chamber aqueous humor ascorbate increased in the eye receiving topical vanadate, and this was compatible with a decreased rate of aqueous humor flow. Topical vanadate did not alter anterior chamber aqueous humor protein or cyclic AMP. In five monkeys intraocular pressure was also significantly reduced by topical vanadate.

Key words: vanadate, intraocular pressure, rabbit, monkey, aqueous humor flow, outflow facility, episcleral venous pressure

Vanadate has been described as a potent inhibitor of the enzyme sodium-potassium-activated adenosine triphosphatase ((Na⁺, K⁺) ATPase). This enzyme has been demonstrated in the rabbit ciliary epithelium and has been postulated to play a role in the production of aqueous humor. Inhibitors of (Na⁺, K⁺) ATPase have been reported to decrease the rate of aqueous humor formation. Vanadate has also been reported to activate adenylate cyclase and thus increase the concentration of adenosine 3',5'-cyclic monophosphate (cyclic AMP). Exogenous catecholamines, which lower intraocular pressure, increase the level of cyclic AMP in the aqueous humor, and this is associated with an increased facility of outflow. The present paper describes the reduction of intraocular pressure following the topical administration of vanadate to the eyes of rabbits and monkeys.

Materials and methods

Vanadate as sodium metavanadate (Na₃VO₄) or sodium orthovanadate (Na₅VO₃) was prepared in distilled water just prior to topical ocular delivery. The solutions were adjusted to a pH between 7.0 and 8.0 with 1N hydrochloric acid. Unanesthetized male albino rabbits (1.5 to 2.5 kg) were restrained in a cloth wrap, and 0.05 ml of the solution was applied to one eye randomly with the diluent administered to the fellow control eye. Male rhesus monkeys (3.5 to 4.5 kg) were subjected to similar studies, but catalepsia was induced with ketamine hydrochloride, 12 mg/kg intramuscularly. Intraocular pressure was measured with a manometrically calibrated pneumotonometer, with topical 0.5% proparacaine hydrochloride used as the anesthetic agent. Tonography was performed with an Alcon EDT-103 tonography unit with the use of the same topical anesthetic agent. Episcleral venous pressure was determined with a 3 mm applanating head attached to a force-displacement transducer and mounted on a...
Table I. Effect of NaVO₃ on intraocular pressure in rabbits

<table>
<thead>
<tr>
<th>Concentration</th>
<th>No. of eyes</th>
<th>Mean intraocular pressure (mm Hg) ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>2.0% Diluent</td>
<td>8</td>
<td>20.5 ± 1.1</td>
</tr>
<tr>
<td>1.0% Diluent</td>
<td>16</td>
<td>19.9 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>23.0 ± 0.5</td>
</tr>
</tbody>
</table>

*Significant difference between eye treated with NaVO₃ and fellow diluent-treated control eye, paired t test, p < 0.005.

Table II. Effect of Na₃VO₄ on intraocular pressure in rabbits

<table>
<thead>
<tr>
<th>Concentration</th>
<th>No. of eyes</th>
<th>Mean intraocular pressure (mm Hg) ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>2.0% Diluent</td>
<td>8</td>
<td>16.5 ± 1.1</td>
</tr>
<tr>
<td>1.0% Diluent</td>
<td>8</td>
<td>16.2 ± 1.1</td>
</tr>
<tr>
<td>0.5% Diluent</td>
<td>8</td>
<td>17.2 ± 1.2</td>
</tr>
<tr>
<td>0.3% Diluent</td>
<td>8</td>
<td>16.6 ± 1.1</td>
</tr>
</tbody>
</table>

Significant difference between eye treated with Na₃VO₄ and fellow diluent-treated control eye, paired t test: *p < 0.001; *p < 0.005; *p < 0.01.

Results

Topical administration of NaVO₃ (Table I) or Na₃VO₄ (Table II) to rabbit eyes resulted in reductions of intraocular pressure related to the dose administered. The mean intraocular pressure was significantly lower after 60 min in eyes treated with NaVO₃ (1% or 2%) or Na₃VO₄ (0.3%, 0.5%, 1.0%, or 2.0%), and the reductions persisted for at least 240 min. The decrease in intraocular pressure was similar (Student’s t test, p > 0.2) during the 240 min following 0.5%, 1%, and 2% Na₃VO₄ or 1% and 2% NaVO₃. Ocular irritation occurred only following the administration of the 2% solutions, but even at this concentration, slit-lamp examination showed no cells or flare in the anterior chamber.

Tonography after the random unilateral administration of 1% vanadate confirmed the reduction of intraocular pressure in 12 rabbits. Intraocular pressure in the treated eye (15.8 ± 1.0 mm Hg, mean ± S.E.M.) was significantly (p < 0.025) lower than in the
Table III. Effect of Na₃VO₄ on aqueous humor ascorbate, protein, and cyclic AMP in nine rabbits*

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Ascorbate (mg/dl ± S.E.M.)</th>
<th>Protein (mg/dl ± S.E.M.)</th>
<th>Cyclic AMP (nM ± S.E.M.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anterior chamber</td>
<td>Posterior chamber</td>
<td>Anterior chamber</td>
</tr>
<tr>
<td>0.5%</td>
<td>25.5 ± 2.0</td>
<td>41.1 ± 2.3</td>
<td>0.72 ± 0.13</td>
</tr>
<tr>
<td>Diluent</td>
<td>25.0 ± 1.9</td>
<td>34.0 ± 3.0</td>
<td>0.68 ± 0.08</td>
</tr>
</tbody>
</table>

*Aqueous humor was obtained 2 hr after topical Na₃VO₄.
†Significant difference between eye treated with Na³VO₄ and fellow diluent-treated control eye, paired t test, p < 0.01.

Control eye (18.5 ± 0.5). Outflow facility was similar (p > 0.5) in vanadate-treated eyes (0.28 ± 0.02 μl/min/mm Hg) and control diluent-treated eyes (0.29 ± 0.02). The average baseline episcleral venous pressure (12.0 ± 0.3 mm Hg) was not altered significantly (p > 0.1) 2 hr after vanadate (11.1 ± 0.5 mm Hg). With the equation F = (P₀ − Pᵥ)C, aqueous humor flow averaged 1.9 μl/min in control eyes and 1.3 μl/min in vanadate-treated eyes. This represented a mean decrease 2 hr after topical 1% vanadate of approximately 30%.

Anterior chamber aqueous humor ascorbate concentrations (Table III) were symmetrical 2 hr after unilateral topical 0.5% Na₃VO₄ in nine rabbits which showed a significant decrease in intraocular pressure (17.0 ± 0.9 vs. 12.6 ± 0.7 mm Hg). However, posterior chamber aqueous ascorbate was significantly (p < 0.01) higher in the Na₃VO₄-treated eyes. The increased posterior chamber ascorbate was compatible with a decreased entry of water in the posterior chamber. Since the new anterior chamber steady-state value for ascorbate may not have been reached at this time, the change in kₑ/kₐₑ could only be approximated at about 40%.

Mean aqueous humor protein was not significantly (p > 0.7) different in the eyes treated with 0.5% Na₃VO₄ in comparison to the fellow diluent-treated eyes (Table III). Anterior chamber aqueous humor cyclic AMP was not significantly (p > 0.7) altered 2 hr following unilateral administration of 0.5%, Na₃VO₄ (Table III).

Baseline intraocular pressures in five monkeys were 17.4 ± 1.0 mm Hg in the experimental eyes and 17.9 ± 1.0 in the control eyes. Intraocular pressure was significantly (p < 0.01) reduced 1, 2, and 3 hr following topical delivery of 0.5% Na₃VO₄. After 3 hr the mean intraocular pressures were 12.6 ± 1.0 mm Hg in the vanadate-treated eyes and 17.4 ± 1.1 in the diluent-treated fellow eyes.

Discussion

The present study demonstrates a lowering of intraocular pressure in rabbits following the topical administration of vanadate as NaVO₃ or Na₃VO₄. The reduction in intraocular pressure persists for at least 360 min, and there appears to be no effect on the opposite eye. A similar lowering of intraocular pressure occurs unilaterally in topically treated normal monkey eyes. Systemic drug absorption and a systemic action do not appear to be significant factors in the decreased intraocular pressure. The fall in intraocular pressure is not associated with significant changes in outflow facility or episcleral venous pressure and may be presumed to be largely due to an effect on aqueous humor production. Both tonographic and aqueous humor ascorbate data in rabbits are compatible with this conclusion.

The anomalous kinetics of (Na⁺, K⁺) ATPase activity from assays with “Sigma Grade” ATP from Sigma Chemical Co., St. Louis, Mo. are reported to result from an inhibitor in the ATP. This inhibitor proves to be the ubiquitous trace metal vanadate and is a potent (Na⁺, K⁺) ATPase inhibitor. The enzyme (Na⁺, K⁺) ATPase spans the plasma membrane of animal cells and maintains a high intracellular potassium/sodium ratio by coupling movement of these ions to the hydrolysis of ATP. Cardiac glycosides inhibit the (Na⁺, K⁺) ATPase by binding to the part.
of the enzyme facing the extracellular fluid. Vanadate appears to inhibit the enzyme from of the enzyme facing the extracellular fluid. It has been shown to decrease the rate of aqueous humor formation in rabbits, cats, and humans. These agents are not effective following topical administration. Lowering intraocular pressure requires systemic administration of doses of cardiac glycosides in the toxic range. Intravitreal injections of ouabain in rabbits produce a unilateral decrease in intraocular pressure. This is associated with a significant increase in posterior chamber ascorbate concentration and unaltered outflow facility. The rabbit findings following intravitreal ouabain are similar to those observed after topical vanadate.

Vanadate causes marked stimulation of adenylate cyclase activity in isolated membrane prepa-"rations. This is of interest because cyclic AMP or its analogs increase outflow facility following intracameral injection. The absence of increased outflow facility or elevation of aqueous humor cyclic AMP following topical administration of Na,VO_4 implies that the ocular effect of vanadate is not related to adenylate cyclase stimulation. However, vanadate also blocks cyclic AMP-induced stimulation of sodium and water transport in amphibian epithelia. This suggests a site of action beyond cyclic AMP generation which may reduce the production of aqueous humor.

The mechanism by which topical vanadate lowers intraocular pressure, presumably by inhibition of aqueous humor secretion, is still unknown. It is tempting to speculate that topical vanadate is acting as an inhibitor of ciliary body (Na^+, K^+) ATPase. In vitro studies are in progress to investigate this action of vanadate. Since traces of vanadate occur widely in animal tissues, it may constitute "a natural mechanism" for regulation of (Na^+, K^+) ATPase activity in vivo.

REFERENCES