References


The Effect of Vanadate on Aqueous Humor Dynamics in Cynomolgus Monkeys

Steven M. Podos, Ping-yu Lee, Colette Severin, and Thomas Mitrag

Topical administration of 1% vanadate in a formulation designed to enhance penetration lowered intraocular pressure in monkeys' eyes. The decrease in intraocular pressure was associated with significant decreases in aqueous humor flow. Tonographic outflow facility was unaltered by topical vanadate. Invest Ophthalmol Vis Sci 25:359–361, 1984

Vanadate, given either as sodium metavanadate (NaVO3) or sodium orthovanadate (Na3VO4), lowers intraocular pressure equally in rabbits.1 The fall in intraocular pressure is not associated with significant changes in outflow facility or episcleral venous pressure. A reduction in the rate of aqueous humor secretion, resulting from the inhibition of ciliary epithelium (Na+K+)ATPase by vanadate, could explain these findings.2 On the basis of the results of tonographic studies, the calculated aqueous humor flow decreases approximately 30% in rabbits 2 hours after topical administration of 1% vanadate.3 Direct measurements of the effect of vanadate on aqueous flow have not been reported.

The topical application of 0.5% Na3VO4 was also reported to lower intraocular pressure in rhesus monkeys.1 In initial trials, we found inconsistent effects of NaVO3 at the maximum soluble concentration of 1% on the intraocular pressure of cynomolgus monkeys and poor penetration of NaVO3 into rabbit eyes.

We now report the effects of NaVO3 prepared with agents to enhance penetration, dimethylsulfoxide (DMSO) and Tween 80 on intraocular pressure, outflow facility measured by tonography, and aqueous humor flow directly measured by fluorophotometry4 in cynomolgus monkeys.

Materials and Methods. Eight adult cynomolgus monkeys, 4–5 kg, were studied. The monkeys were kept in primate chairs throughout each experiment. A drop of a local anesthetic (0.5% proparacaine hydrochloride) was applied to the eye before all measurements. The intraocular pressure was measured in awake animals, and the outflow facility and aqueous humor flow were measured in animals anesthetized with ketamine hydrochloride, 5–10 mg/kg given intramuscularly.

Vanadate, as sodium metavanadate (NaVO3) (E. Merck, Darmstadt, Germany), was prepared in distilled water with 10% DMSO and 5% Tween 80 just prior to topical ocular delivery. Solutions were adjusted to a pH between 7.0 and 8.0 with 1 N hydrochloric acid. For all experiments, two 50-μl drops of 1% vanadate, 3–5 min apart, were applied to either the right or left eye, chosen at random. An equal volume of the same diluent containing DMSO and Tween 80 was administered to the fellow control eye. Baseline intraocular pressure was measured with a calibrated pneumotonometer twice prior to administration of the drops. Repeat intraocular pressure measurements were made at 30, 60, 120, 240, and 360 min after administration of 1% vanadate.

Tonography was performed with an Alcon EDT-103 tonography unit. Baseline outflow facility was determined from 9 AM to 10 AM. The tonography results were obtained at 2 hours after administration of 1%
Taken 1-3 hrs after instillation of the vanadate. The tralateral eye. Fluorophotometric measurements were by the reference filter reading, and the ratio (F) was cornea and anterior chamber readings were divided between the absolute values of the slopes of the anterior chamber and corneal lines of best fit. The value of $F_c/F_a$ was determined from the corresponding lines of best fit at 2 hrs after vanadate administration. Values of 106 µl for anterior chamber volume⁶ and 50 µl for cornea volume (M. E. Yablonski and J. B. Serle, unpublished data) were used in the calculations. Statistical significance was determined by the pared t test. These experiments adhered to the ARVO resolution on the use of laboratory animals in research.

Results. Topical administration of 1% vanadate to monkey eyes resulted in reductions of intraocular pressure (Table 1). The mean intraocular pressure was significantly ($P < 0.05$) reduced in the time period 120-240 min after vanadate administration. Ocular irritation was not observed in any of the monkey eyes at any time after topical application of 1% vanadate.

Tonographic examination 2 hours after the unilateral administration of 1% vanadate confirmed the reduction of intraocular pressure in eight monkeys. Intraocular pressure in the treated eye (18.1 ± 0.6 mmHg, mean ± SE) was significantly ($P < 0.005$) lower than in the fellow control eye (21.5 ± 0.7 mmHg). Outflow facility (Table 2) was similar ($P > 0.5$) in vanadate-treated eyes and control diluent-treated eyes.

Two hours after 1% vanadate administration, the aqueous humor flow (Table 2) in the treated eyes (1.36 ± 0.13 µl/min, mean ± SE) was significantly ($P < 0.005$) lower than in the fellow control eyes (1.89 ± 0.18 µl/min). Baseline aqueous humor flow was similar ($P > 0.8$) in the treated and fellow control eyes.

Discussion. In this study, two drops of 1% vanadate prepared with agents to enhance penetration applied to monkey eyes produced a significant unilateral reduction of intraocular pressure. No alteration of outflow facility was demonstrated. The reduction of intraocular pressure was associated with a 30% reduction in aqueous humor flow as measured by fluorophotometry. One may calculate from the Goldmann flow equation, $F = C(Po - Pe)$, that there is a 33% reduction of aqueous flow at 2 hours after vanadate administration. These findings are consistent with previous studies¹⁻³ in rabbits in which the decrease in intraocular pressure after vanadate administration was not associated with significant changes in outflow facility or episcleral venous pressure and was associated with an

### Table 1. The effect of 1% vanadate on intraocular pressure in eight awake monkeys

<table>
<thead>
<tr>
<th>Mean intraocular pressure ± SE (mmHg)</th>
<th>0 min</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
<th>240 min</th>
<th>360 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vanadate</td>
<td>19.5 ± 0.4</td>
<td>19.5 ± 0.4</td>
<td>18.6 ± 0.7</td>
<td>17.9 ± 0.6$^*$</td>
<td>18.0 ± 0.7$^*$</td>
<td>18.9 ± 0.5</td>
</tr>
<tr>
<td>Diluent</td>
<td>19.6 ± 0.4</td>
<td>20.0 ± 0.4</td>
<td>20.0 ± 0.3</td>
<td>19.9 ± 0.3</td>
<td>20.0 ± 0.3</td>
<td>19.9 ± 0.4</td>
</tr>
</tbody>
</table>

* Significant difference between eyes treated with vanadate and fellow control eyes, paired t test, $P < 0.05$.

### Table 2. The effect of 1% vanadate on aqueous humor dynamics in eight monkeys under ketamine anesthesia*

<table>
<thead>
<tr>
<th>Intraocular pressure (mmHg)</th>
<th>Outflow facility (µl/min/mmHg)</th>
<th>Aqueous flow (µl/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vanadate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>22.4 ± 1.0</td>
<td>0.51 ± 0.05</td>
</tr>
<tr>
<td>Treated</td>
<td>18.1 ± 0.6$^+$</td>
<td>0.52 ± 0.04</td>
</tr>
<tr>
<td>Diluent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>22.9 ± 1.3</td>
<td>0.55 ± 0.06</td>
</tr>
<tr>
<td>Treated</td>
<td>21.5 ± 0.7</td>
<td>0.58 ± 0.08</td>
</tr>
</tbody>
</table>

* Values shown are means ± SE before and 120 min after treatment.

$^+$ Significantly different as compared with baseline ($P < 0.02$) and to control eyes ($P < 0.005$), paired t test.

$^*$ Significantly different compared with baseline and with control eyes, paired t-test. $P < 0.005$. 

vanadate. Tonography values were approximated from the 1955 Friedenwald tables.

A previously described fluorophotometric method was utilized to measure aqueous humor flow.⁵ The fluorescein iontophoresis was done at 4 PM and fluorescence measurements were made from 9 AM to 12 PM on the following day. The iontophoresis was carried out in the central 4 mm of the cornea with an electrode of 10% fluorescein in 2% agar. A 200 µA current was used for 5 min. Fluorophotometric measurements of the cornea and anterior chamber were repeated at about 45-min intervals. Following these baseline measurements, 1% vanadate was applied topically to one eye of each animal at about 12:30 PM on the same day. An equal volume of diluent was applied to the contralateral eye. Fluorophotometric measurements were taken 1-3 hrs after instillation of the vanadate. The cornea and anterior chamber readings were divided by the reference filter reading, and the ratio (F) was recorded. For each animal, the natural logarithm of F was plotted against time. The lines of best fit and their slopes were calculated by the least-squares method. The value of A used for each eye was midway the absolute values of the slopes of the anterior chamber and corneal lines of best fit. The value of $F_c/F_a$ was determined from the corresponding lines of best fit at 2 hrs after vanadate administration.
increase in posterior chamber aqueous humor ascorbate, indicative of decreased entry of water into the posterior chamber. Thus, the effect of vanadate may be presumed to be largely due to an effect on aqueous humor production.

(Na⁺K⁺)ATPase activity is present in ciliary epithelium. Inhibitors of this enzyme, such as ouabain given systemically, lower the rate of aqueous humor formation in cats and humans. Vanadate is also a potent inhibitor of (Na⁺K⁺)ATPase, which acts in a fashion different from the cardiac glycosides. Although vanadate appears to have little or no effect on human intraocular pressure when administered topically in aqueous solutions, formulations to enhance penetration, as in the current monkey study, may produce positive results. Ocular penetration and (Na⁺K⁺)ATPase studies currently are being performed to elucidate the vanadate mode of action.

Vanadate also is noted to stimulate adenylate cyclase activity in isolated membrane preparations. Preliminary experiments indicate that vanadate also stimulates monkey ciliary body-iris adenylate cyclase in vitro (unpublished data, T. Mittag). Cyclic AMP and its analogs are reported to increase outflow facility. The absence of increased outflow facility and the elevation of aqueous humor cyclic AMP in rabbits after topical administration of vanadate implies that in rabbits the ocular effect of vanadate is not related to adenylate cyclase stimulation. However, the recent report that forskolin, an agent that stimulates adenylate cyclase directly without affecting the cell membrane receptors that are coupled to the cyclase enzyme, lowers intraocular pressure in rabbits, monkeys, and humans and reduces aqueous flow in rabbits suggests that reduction of aqueous humor formation may be another mechanism whereby stimulation of this enzyme could affect intraocular pressure.

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

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References

Neovascularization of the Iris: An Experimental Model in Cats

Einar Stefansson, Maurice B. Landers III, Myron L. Wolbarsht, and Gordon K. Klintworth

Neovascularization of the iris was induced in cats by removing the vitreous and lens and creating a rhegmatogenous retinal detachment. The presence of new blood vessels on the anterior surface of the iris was verified from the second month onward by slit lamp examination, as well as by light microscopy six to twelve months after the operation. Control eyes undergoing vitrectomy and lensectomy, but without retinal detachment, did not develop rubeosis iridis. This model may allow investigation into causes and therapy of rubeosis iridis. Invest Ophthalmol Vis Sci 25:361-364, 1984

The introduction of vitrectomy for the treatment of vitreal and retinal complications of diabetes mellitus and other conditions has rekindled interest in iris neovascularization, since vitrectomy apparently predis-