The Ocular Hypotensive Effects of Demeclocycline, Tetracycline and Other Tetracycline Derivatives

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Demeclocycline, tetracycline and other tetracycline derivatives lowered intraocular pressure (IOP) in rabbits following intravitreal injection, but the onset of this effect was not evident until 1 or more days after drug administration. Of the drugs tested, demeclocycline was the most active ocular hypotensive agent. Demeclocycline caused a dose-dependent decrease in IOP. The maximum IOP decrease of approximately 12 mm Hg occurred 5 days after intravitreal administration of 0.5 mg, with the effect persisting for over a week. Demeclocycline did not alter tonographically measured aqueous humor outflow facility or episcleral venous pressure. Based on calculated aqueous humor flow rates following 0.2 mg demeclocycline, a 62% decrease in aqueous humor formation occurred 7 days after intravitreal injection. The flow-to-diffusion ratio for ascorbate was reduced 54% 6 days after the intravitreal administration of demeclocycline, a change also consistent with suppression of aqueous humor formation. Anterior chamber aqueous humor protein concentration was increased 6 days after demeclocycline administration. No histologic changes were present in the treated eyes by light microscopy. Intravitreal demeclocycline similarly lowered IOP in cats, with the duration of effect lasting up to 20 days. Invest Ophthalmol Vis Sci 30:1594-1598, 1989

Possible roles in the regulation of intraocular pressure (IOP) for biologically active peptides, either those present locally within peptide containing nerve fibers or those reaching the eye through the blood, remain poorly defined. Intravenous administration of the nanopeptide vasopressin, or antidiuretic hormone (ADH), stimulates aqueous humor formation and elevates IOP in rabbits, apparently as the result of a direct effect on active sodium transport across the ciliary epithelium. Intravenous desmopressin, a synthetic analog of vasopressin, similarly increases aqueous humor formation and raises IOP. In its antidiuretic action on the kidney, both vasopressin and desmopressin stimulate the production of the intracellular second messenger hormone, cyclic adenosine 3'5' monophosphate (cyclic AMP), which initiates a series of events that ultimately decrease urine flow. The tetracycline drugs inhibit the vasopressin-induced stimulation of cyclic AMP in the kidney and thus block the antidiuretic action of vasopressin. The most active tetracycline derivative in this regard is demeclocycline, which may even induce nephrogenic diabetes insipidus. Demeclocycline is an effective therapy for the syndrome of inappropriate ADH secretion because of this effect.

In the eye, vasopressin does not stimulate cyclic AMP production in isolated ciliary processes, and desmopressin does not elevate aqueous humor cyclic AMP. Paradoxically, however, we have found that demeclocycline inhibits the IOP rise caused by desmopressin. Demeclocycline alone shows no acute effect on IOP; but during the course of our studies, we observed that demeclocycline has an ocular hypotensive effect developing days after local administration to the eye. We therefore studied the direct effects of demeclocycline, tetracycline and other tetracycline derivatives on IOP.

Materials and Methods

Awake, 2- to 3-kg albino rabbits and 3- to 4-kg cats were restrained in cloth wrappers or in Plexiglas boxes specially designed for each species. Systemic anesthetic agents were not used during any of the experiments. IOP was measured after 0.5% topical proparacaine HCl anesthesia using a Digilab Model 30R pneumotonometer (Cambridge, MA) manometrically calibrated for rabbit and cat eyes. Drugs for intravitreal injection were dissolved in filtered (0.22 μm) distilled water or normal saline. The pH was adjusted with NaOH as close to neutrality as possible.
without drug precipitation. An acidic solution was required for the 0.2 mg (pH 6.6) and 0.5 and 1.0 mg (pH 6.3) injections. Osmolarity was adjusted with NaCl to between 350 and 360 mOsm.

Intravitreal injections were performed after topical 0.5% proparacaine HCl anesthesia using a 30-gauge needle on a 0.025 ml Hamilton syringe with a Chaney adaptor. The final volume injected was 0.01 ml, except for the 1.0 mg dose of demeclocycline which required 0.02 ml. In all rabbits, one eye received intravitreal drug and the fellow control eye received an equal volume of intravitreal filtered sterile saline with an adjusted pH similar to the drug solution. In the cats, each animal received a single unicoic injection of either drug or saline. Except where indicated, the rabbits but not the cats also received 10 mg/kg intraperitoneal indomethacin (40 mg/ml in 1 M Na2CO3) 2 to 3 hr before intravitreal injection. A stable baseline IOP was established both before indomethacin pretreatment and again before intravitreal drug injection. After drug administration, IOP generally was measured hourly for 6 hr and then every 24 or 48 hr until it returned to baseline. To control for possible diurnal fluctuations, daily IOP readings were recorded between 1 and 3 PM.

Aqueous humor dynamics were studied in rabbits following intravitreal injection of 0.2 mg demeclocycline. Seven days after demeclocycline administration in six animals, aqueous humor outflow facility was measured on awake and restrained animals by tonography (4 min recordings) using an electronic Schiötz tonometer connected to a graph recorder. Aqueous humor flow rate was calculated using the Goldmann equation, \[ F = (P_o - P_v) C \]; where \( F \) = aqueous humor flow rate in \( \mu l/min \), \( P_o \) = intraocular pressure in mm Hg, \( P_v \) = episcleral venous pressure in mm Hg, and \( C \) = aqueous humor outflow facility in \( \mu l/min/mm Hg \). Episcleral venous pressure was measured with an episcleral venomanometer in six rabbits before and 7 days following intravitreal demeclocycline.

Six days after intravitreal injection of 0.2 mg demeclocycline in another 15 awake rabbits, aqueous humor samples were obtained by posterior and anterior chamber paracentesis after topical 0.5% proparacaine HCl anesthesia. These aqueous humor samples were placed immediately in 4.0% metaphosphoric acid and titrated with dichlorphenol-indophenol for estimation of ascorbate concentration. The ascorbate ratio of the flow coefficient (\( k_{oa} \)) to the diffusion coefficient (\( k_{dp} \)) was calculated from the Kinsey-Palm formula, \[ k_{oa}/k_{dp} = (d \times C_o - C_p)/(C_h - C_o) \] \[ (1) \]

where \( d \) is the Donnan factor, \( C \) the ascorbate concentration in the anterior chamber (\( C_a \)) or posterior chamber (\( C_h \)) aqueous humor and in the plasma (\( C_p \)). Assuming that the rate of diffusion remains constant, this relationship has been employed to measure the effects of various agents on the flow rate. The protein concentration of these anterior chamber aqueous samples also was measured using Biuret and Folin phenol reagents (Total Protein Kit No. 690, Sigma Chemical Co., St. Louis, MO).

For histopathologic study, three rabbits were sacrificed with an overdose of sodium pentobarbital 6 days after intravitreal injection of 0.2 mg demeclocycline. Both the drug and saline injected eyes were fixed in formalin, embedded in paraffin, serial-sectioned in four quadrants, and stained with hematoxylin/eosin or with Periodic Acid Schiff.

The effects on IOP of six additional tetracycline compounds were tested after intravitreal administration of 0.5 mg in rabbits using the same protocol, except that no indomethacin pretreatment was given. IOP was measured daily in these rabbits for 14 days.

Intravenous demeclocycline, 40 mg/kg, was administered via the marginal ear vein in eleven rabbits. IOP was measured at 3 hr and 6 hr following injection and then daily for a total of 8 days.

Statistical analysis used the student t-test on paired differences to compare drug- and vehicle-treated eyes within each experimental group and an unpaired t-test for intergroup analysis. Drug effects are reported as mean ± SEM, with \( P < 0.05 \) considered significant. This study conformed to the ARVO Resolution on the Use of Animals in Research.

**Results**

Intravitreal injection of demeclocycline in rabbits resulted in a dose-dependent decrease in IOP (Fig. 1). At the 0.1 mg dose, a statistically significant decrease
Table 1. Ocular effects of intravitreal demeclocycline (0.2 mg) in rabbits

<table>
<thead>
<tr>
<th>Drug-treated eye</th>
<th>Vehicle-treated eye</th>
<th>No. of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraocular pressure (mm Hg)</td>
<td>11.1 ± 1.6*</td>
<td>18.5 ± 1.7</td>
</tr>
<tr>
<td>Aqueous humor outflow facility (μl/min/mm Hg)</td>
<td>0.21 ± 0.01</td>
<td>0.21 ± 0.02</td>
</tr>
<tr>
<td>Episcleral venous pressure (mm Hg)</td>
<td>7.2 ± 1.9</td>
<td>8.1 ± 2.1</td>
</tr>
<tr>
<td>Calculated aqueous humor flow (μl/min)</td>
<td>0.82*</td>
<td>2.18</td>
</tr>
<tr>
<td>Aqueous protein (mg/dl)</td>
<td>139.8 ± 15.9*</td>
<td>64.4 ± 8.8</td>
</tr>
</tbody>
</table>

Mean ± SEM; t-statistics on the paired differences for drug treated vs. vehicle treated eye; *P < 0.05.
† Calculated aqueous humor flow rate, see text.
‡ Kinsey-Palm flow to diffusion ratio, see text.
Aqueous humor dynamics were studied 7 days after intravitreal injection.

Discussion

Demeclocycline, tetracycline and other tetracycline derivatives effectively lower IOP in rabbits after intravitreal injection. The IOP effect develops 1 or more days after administration for all agents. Demeclocycline is the most effective ocular hypotensive agent of the drugs tested, and we studied its action in greater detail. Intravitreal demeclocycline causes a dose-dependent decrease in intraocular pressure. This effect is profound, delayed several days in onset after intravitreal injection, and persists for over a
Table 2. Effects of intravitreal tetracyclines on intraocular pressure in rabbits

<table>
<thead>
<tr>
<th>Compound</th>
<th>Max effect* (mm Hg, mean ± SEM)</th>
<th>Day of lowest IOP†</th>
<th>Day of onset‡</th>
<th>Duration of effect (days)§</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demeclocycline</td>
<td>11.6 ± 1.2</td>
<td>6.9 ± 1.3</td>
<td>2</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>7.9 ± 0.7</td>
<td>5.6 ± 1.1</td>
<td>1</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>7.8 ± 0.5</td>
<td>2.5 ± 0.5</td>
<td>1</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Minocycline</td>
<td>6.1 ± 1.1</td>
<td>4.0 ± 0.6</td>
<td>2</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Methacycline</td>
<td>3.9 ± 0.9</td>
<td>6.3 ± 1.1</td>
<td>7</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Chlortetracycline</td>
<td>4.3 ± 1.5</td>
<td>3.2 ± 1.0</td>
<td>—</td>
<td>—</td>
<td>6</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>4.3 ± 1.4</td>
<td>2.8 ± 0.7</td>
<td>—</td>
<td>—</td>
<td>6</td>
</tr>
</tbody>
</table>

* Maximum decrease in IOP for each animal: IOP control minus IOP treated eye.
† Mean day of greatest IOP effect.
‡ First day the mean IOP in the treated eyes was significantly lower (P < 0.05) than the mean IOP in the control eyes, using t-statistics on paired data.
§ Number of days mean IOP was significantly lower (P < 0.05) in the treated than in the control eye, using t-statistics on paired data.

week. The tonographic data and aqueous humor ascorbate ratios indicate that demeclocycline decreases IOP by suppressing aqueous humor formation. In addition, the increase in aqueous humor protein is also consistent with the decrease in aqueous humor formation. No untoward effects such as inflammation were observed during these studies, and histologic examination of three eyes at the time of maximum IOP drop revealed no evident pathology by light microscopy. A similar ocular hypertensive effect occurs in cats following intravitreal demeclocycline.

The nature of the hypertensive response and the properties of these drugs may explain why their pronounced effects on intraocular pressure have been so long unappreciated. Available antiglaucoma agents act within a few hours, and the onset of the tetracycline-induced ocular hypotension is delayed long beyond the time course of most studies of aqueous humor dynamics. In addition, the tetracyclines as currently formulated penetrate poorly into the eye after topical administration.13,14 In the current studies, we specifically used intravitreal injections to bypass the poor ocular penetration of these agents.

The biochemical mechanism by which tetracyclines decrease intraocular pressure is unclear. The unilaterality of the effect argues against a systemic action, and the ineffectiveness of systemic drug administration in lowering IOP indicates that intraocular drug deposition is critical. While demeclocycline inhibits the acute rise in IOP induced by desmopressin,4 there is no compelling evidence for tonic vasoressin activity on the ciliary epithelium, and the long delay required for tetracyclines to lower IOP suggests an alternative mechanism. Some of the neuropeptides in the eye cause prolonged physiological effects in other systems,1 and tetracyclines may be interacting with an as yet undiscovered neuroendocrine mechanism in the eye. In this regard, the delayed onset of their effect is consistent even with retrograde axoplasmic transport to peripheral ganglia innervating the eye and neural effects outside the eye itself. As antibiotics, tetracyclines act on the bacterial ribosome to suppress protein synthesis15; in higher concentrations they inhibit nucleic acid and protein synthesis by mammalian cells in vitro.16 Tetracyclines thus may inhibit the intraocular synthesis of a peptide or protein critical for ciliary epithelial function. Finally, these agents may have another effect on the ciliary epithelium responsible for decreasing aqueous humor flow and IOP.

Whatever the biochemical mechanism of action, the time course and duration of the tetracycline effect on IOP are unusual. A depot mechanism in the vitreous may account for these observations. Alternatively, if tetracyclines interact with a specific intraocular receptor or modulate local neuroendocrine activity, they may prove useful in defining a novel regulatory mechanism governing intraocular pres-

Fig. 3. The change in intraocular pressure (IOP) following 0.2 mg intravitreal demeclocycline in cats. The values represent (IOPtreated eye) - (IOPcontrol eye) for 11 cats. Using t-statistics on the paired differences, all changes from baseline are significant statistically: P < 0.0005, for days 1 to 9; P < 0.002, for days 10 to 20.
sure, a mechanism registering its effects over a time period of days to weeks rather than minutes to hours.

The tetracyclines, especially demeclocycline, are among the most effective ocular hypotensive agents when studied in normal rabbits and cats. Their prolonged action and apparent lack of adverse ocular side effects suggest possible usefulness for antiglaucoma therapy in man. Clearly, the interactions of tetracyclines and IOP require further study.

Key words: tetracyclines, intravitreal injection, intraocular pressure, aqueous dynamics, rabbits

References