Reduction of intraocular pressure in glaucomatous dogs by a new cholinergic drug

C. Y. Chiou, J. Trzeciakowski,* and K. N. Gelatt

N-Demethylated carbachol (DMC) was synthesized and analyzed for its ocular effects. DMC has been found to penetrate ocular tissue effectively. It has been shown to be free of acute toxic properties in rabbits and dogs at therapeutic doses and has been demonstrated to lower intraocular pressure of glaucomatous beagles without causing intense miosis. The effects of DMC and pilocarpine on outflow facility were compared in normal dogs. At the dose used in these experiments, both drugs affected outflow facility to a similar extent. It is concluded that DMC could be a potentially useful ocular hypotensive agent.

Key words: N-demethylated carbachol, glaucomatous beagle, intraocular pressure, outflow facility, penetrability, ocular toxicity, aqueous humor

Drugs used in the treatment of open-angle glaucoma may be grouped into two major categories based on their primary mode of action: drugs that increase facility of aqueous humor outflow, such as pilocarpine, and those that reduce aqueous humor formation, such as acetazolamide.

Pilocarpine is the drug most frequently used to treat open-angle glaucoma. The usefulness of pilocarpine stems primarily from its ability to penetrate the cornea because its molecule consists of a tertiary nitrogen group instead of a quaternary nitrogen group. Most cholinergic drugs contain quaternary nitrogen groups which limit their penetrability across the biological barrier.

Unfortunately, pilocarpine is not a perfect drug for the treatment of glaucoma because it possesses some undesirable qualities such as short duration of action, miosis, and local irritation. Thus other drugs which could be better than pilocarpine or serve as alternatives in glaucoma therapy have been sought.

N-Demethylated carbachol (DMC) is a tertiary nitrogen compound possessing cholinergic effects equipotent to pilocarpine in various autonomic preparations in vivo and in vitro. Its ability to lower the intraocular pressure (IOP) of glaucomatous dogs has been evaluated and reported in this communication.

Methods

Materials. DMC was synthesized by the method described by Trzeciakowski and Chiou and Haz-
aid et al. 3 H-DMC was prepared by ICN Pharmaceuticals, Inc., and purified with thin-layer chromatography on silica gel with a solvent system of chloroform and acetone (3:1). The specific activity of 3H-DMC was 31 mCi/mmol. It was shipped and stored in powder form. Solutions were made fresh immediately before use to minimize the exchange of 3H with the solvent. Pilocarpine nitrate was obtained commercially.

**Intraocular pressure (IOP) and pupil size.** Glaucomatous beagles were tested to determine the effect of DMC on IOP which was measured with the Mackay-Marg electronic applanation tonometer (Berkeley Bio-Engineering, San Leandro, Calif.). Two drops of 0.4% benoxinate (Dorsacaine; Dorsey Laboratories) were applied to each eye prior to pressure determination. Pupil size was measured with calipers under uniform artificial illumination.

Baseline values of IOP and pupil size were determined for 3 successive days. Drug treatment followed for the next 5 days. For drug treatment, 50 μl of a sterile phosphate-buffered saline, pH 7.4, plus DMC solution was administered randomly to one eye of each animal; the other eye received 50 μl of plain phosphate-buffered saline as a control. IOP and pupil size were recorded hourly for 6 hr after drug application and compared with baseline values. The paired t test was performed for statistical analysis of data. Consequently, no standard deviation of pooled data was provided in Figs. 1 through 3 in order to avoid confusion.

**Penetration of DMC into eyes.** A 25 μl volume of phosphate-buffered saline solution of 3H-DMC (0.128M, 31 mCi/mmol) was applied to the New Zealand albino rabbit (2 kg body weight) eye, and the eyelids were held closed for 1 min. At 10, 20, 60, and 180 min after application, rabbits were sacrificed with the injection of a bolus of air into the marginal ear vein. The eye was rinsed with saline to remove residual surface radioactivity and blotted. A 100 to 150 μl volume of aqueous humor was withdrawn with a syringe, and the cornea, iris, and ciliary body were removed by dissection. Tissues were weighed in counting vials, digested with 0.5 ml of NCS solubilizer (Amersham/Searle Corp.), and counted in 10 ml of scintillation solution (Biofluor; New England Nuclear Corp.) with a scintillation counter (Model LS 100; Beckman Instruments, Inc.). Blanks and quenchings were determined with aqueous humor and tissue samples from control rabbit eyes.

**Outflow facility.** Mongrel dogs weighing 13 to 18 kg were anesthetized with thiamylal (8 mg/kg

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**Fig. 1.** Time-response relationship of DMC effects on IOP in glaucomatous beagles for 5 days. DMC was instilled in volumes of 50 μl, once per day for 5 days. Statistical analysis was performed with paired Student t test. Each point is an average value of all animals (n = number of animals used) from 3-day average readings for control and 5-day average readings for treated. A, 4% DMC. B, 6% DMC. C, 8% DMC. Control baseline values determined on 3 consecutive days; o, treatment values recorded on 5 consecutive days; asterisk and double asterisks, significantly different from control values at p < 0.05 and p < 0.01, respectively.
A tracheal tube was inserted, and anesthesia was maintained with 1% methoxyflurane. For perfusion, the anterior chamber of the eye was cannulated and attached to a pressure transducer and microliter delivery system. A second cannulation was performed for drug injection. The eye was perfused with a buffered solution (NaCl 137 mM, KCl 4.7, CaCl₂ 1.53, MgCl₂ 0.67, Na₂HPO₄ 0.49, NaH₂PO₄ 0.11, and glucose 5.55) and allowed to stabilize, and the normal IOP (P₀) recorded on a polygraph. The perfusion rates necessary to maintain the eye at pressures 5 mm Hg (P₁) and 15 mm Hg (P₂) above P₀ over a 4 min period were then determined. Four control readings of the flow were then calculated to obtain values for the outflow facility (C).

The outflow facility was calculated from the equation:

\[ C = \frac{(F₂ - F₁)}{(P₂ - P₁)} \]

where \( F₂ \) and \( F₁ \) were the rates of perfusion at IOPs of \( P₂ \) and \( P₁ \), respectively.⁹

Since each flow measurement took 4 min, it required 16 min to determine the control outflow facility. After a control value of C was determined, 10 μl of DMC, pilocarpine, or saline solutions were injected intracameral. Twenty minutes after injection, the flow rates required to maintain IOP at the previously determined values of \( P₁ \) and \( P₂ \) were redetermined exactly as for the controls. Thus C values were obtained during the 16 min period prior to drug (or saline) injection and during the period 20 to 36 min after the injections.

**Ocular toxicity.** Acute ocular toxicity of DMC and pilocarpine was studied on New Zealand white rabbits (1.5 to 2.0 kg) and beagles. The eyes of all animals were stained with fluorescein (Fluor i-strip; Ayerst Labs, Inc.) and examined with a...
slit-lamp biomicroscope (Model 7451; Mentor Corp.) under cobalt blue light. One eye from each animal was selected at random and given 25 μl of topically applied drug or buffer solution daily for a week. The eyes were examined at 1 and 6 hr after drug administration for evidence of irritation and toxicity. A chronic 8-week ocular toxicity study, including gross and histopathologic examinations in the rabbit, will be conducted in the near future.

Results

Effects of DMC on IOP and pupil size. The largest reduction of IOP was caused by a 6% solution of DMC (Fig. 1, A through C). No further decrease in IOP was obtained with the 8% DMC solution (Fig. 1, C). These results, however, were complicated by the fact that the magnitude of the pressure drop was dependent not only on the concentration of DMC administered but also on the initial level of IOP at the time of experiment. The IOP could be lowered to around 25 mm Hg and no further. With regard to duration of the effect, DMC action extended beyond the 6 hr period studied. The IOPs of treated eyes were consistently and significantly lower than the baseline values 24 hr after drug instillation (Fig. 1). When data of day 1 treatment alone were plotted, the IOPs at time 0 for baseline and for treated eyes were the same (Fig. 2).

DMC caused only a moderate miosis. The pupil diameters decreased less than 1 mm (approximately 16%) even with the 8% concentration (Fig. 3, A and B).

Penetration of DMC. The concentration of 3H-DMC in the cornea was highest immediately after application and declined over the next 3 hr (Fig. 4, A).

The drug concentration was 20.3 ± 2.1 (S.E.) pmol/mg in the aqueous humor 20 min after topical administration (Fig. 4, B). On the basis of the aqueous humor volume of 287
Table I. Effects of DMC and pilocarpine on outflow facility

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Control (μl/min/mm Hg)</th>
<th>Experimental (μl/min/mm Hg)</th>
<th>Δ (μl/min/mm Hg)</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMC*</td>
<td>5</td>
<td>0.29 ± 0.021</td>
<td>0.58 ± 0.061</td>
<td>0.29 ± 0.04</td>
<td>101 ± 14</td>
</tr>
<tr>
<td>Pilocarpine</td>
<td>5</td>
<td>0.28 ± 0.03</td>
<td>0.62 ± 0.05</td>
<td>0.35 ± 0.07</td>
<td>140 ± 39</td>
</tr>
<tr>
<td>Sham</td>
<td>2</td>
<td>0.29 ± 0.09</td>
<td>0.30 ± 0.10</td>
<td>0.01 ± 0.01</td>
<td>3 ± 3</td>
</tr>
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*3.7 × 10^-8 mol injected intracameraly.

μl determined for rabbits of similar size and weight, it was calculated that 0.18% of the topical dose of DMC was present in the aqueous humor at 20 min.

The drug levels in the iris (Fig. 4, C) and ciliary body (Fig. 4, D) paralleled those in aqueous humor. The average weights of cornea, iris, and ciliary body were 64.4 ± 1.6, 34.9 ± 1.5, and 27.1 ± 1.5 mg (S.E., n = 19), respectively. From these weights and the volume of aqueous humor, the concentration of DMC in the eye 20 min after application was estimated to be 1.50 × 10^-8 mol. The dose of DMC instilled was 3.2 × 10^-6 mol. Therefore at least 0.46% of the dose was absorbed, since part of the absorbed DMC was metabolized and/or flowed out from Schlemm’s canal.

Effects of DMC on outflow facility. The increase in outflow facility was measured 20 min after the injection of 3.7 × 10^-8 mol DMC or pilocarpine into the anterior chamber (Table I). Sham procedures failed to produce significant increases in outflow facility (Table I), indicating that the increase is induced by drugs and not by the perfusion method.

Ocular toxicity of DMC. DMC was tested for acute irritant and toxic effects on rabbit and beagle eyes. There was little evidence of conjunctival hyperemia and chemosis even at the highest concentration (10%) studied. Examination of the corneas under cobalt blue light after staining with fluorescein revealed that no damage to the epithelium occurred. The irides appeared normal, and aqueous humor remained clear. In all cases the eyes were given a rating of 0 on the scale of Draize et al. Pilocarpine administered to the eyes resulted in evident hyperemia and chemosis. These were most pronounced immediately following the initial drug application but subsided after repeated instillation. Its effect on the iris and aqueous humor was negligible.

Discussion

Pilocarpine has been employed for over a century to reduce IOP of glaucoma patients. It has been found to produce a number of untoward side effects, yet there are few suitable alternatives. Irreversible anticholinesterases such as echothiophate are more potent but are also more toxic. Carbachol does not penetrate the eye well enough to be reliable unless a wetting agent is used.

DMC appeared to be remarkably free of acute irritant and toxic properties in the eye. Pilocarpine, on the other hand, caused marked conjunctival hyperemia and other signs of irritation.

The eyes of rabbits and dogs are more susceptible to damage by toxic substances than are human or monkey eyes. Although the use of rabbits occasionally results in misjudgment of a drug as unsafe for use in human eyes, the opposite is rarely, if ever, true. Therefore the lack of toxicity of DMC in these animals suggests that this drug may be safe for topical use in man.

Twenty minutes after topical application of DMC at pH 7.4, 0.18% of the dose was present in the aqueous humor. This value is quite close to the data for pilocarpine absorption in the literature. Chrai and Robinson found an aqueous humor pilocarpine concentration of 6 × 10^-4 mg/ml 20 min after instillation of 25 μl of 1 × 10^-2M solution at pH 6.24. On the basis of an aqueous humor volume of 287 μl, this is equivalent to 0.25% of the applied dose.

Although the absorption of pilocarpine can be enhanced greatly at pH 7.4, the drug is not very stable at pH higher than 6.0.
Pilocarpine undergoes base-catalyzed epimerization to form inactive isopilocarpine or is converted to pilocarpic acid by cleavage of the lactone ring at high pH. For this reason, most ophthalmic preparations of pilocarpine are adjusted to be within a pH range of 4.5 to 5.5. Between these values, more than 95% of the pilocarpine is in the ionized form and will penetrate the eye less readily than DMC administered at pH 7.4.

DMC reduced IOP significantly in glaucomatous beagles to between 24 and 27 mm Hg. These values are within the range of pressures observed in normal animals. DMC produced only slight miosis at the same doses tested above. If this is true, there would be a distinct advantage to using DMC rather than miotics, particularly in the treatment of glaucoma patients who have cataracts or shallow anterior chambers. Whether or not there is a significant dissociation between ocular hypotensive effects and miotic effects of DMC will be determined in future experiments.

In summary, DMC has been found to lower IOP and yet possess few side effects. Although the penetrability of DMC is lower than that of pilocarpine at pH 7.4, commercial pilocarpine eyedrops are in solution with a pH range of 4.5 to 5.5, at which the penetrability of pilocarpine is even poorer than that of DMC. These results indicate that DMC is a potentially useful ocular hypotensive agent.

Clinical trials of DMC on open-angle glaucoma patients have been conducted recently at the Department of Ophthalmology in the National Taiwan University Hospital by Dr. Por T. Hung and his associates. DMC solutions of 3% and 6% concentrations administered topically once in the morning reduced IOP 30% and 36%, respectively (unpublished observation). The complete clinical study will be published in the near future.

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REFERENCES