**Objective:** To evaluate the effects of WIN 55212-2, a cannabinoid receptor agonist, on intraocular pressure and aqueous humor dynamics in normal monkeys and monkeys with glaucoma.

**Methods:** Intraocular pressure was measured prior to and up to 6 hours after the topical administration of WIN 55212-2 to 1 eye of 5 normal monkeys and to the glaucomatous eye of 8 monkeys with unilateral laser-induced glaucoma. Tonographic outflow facility and fluorophotometric flow rates of aqueous humor were measured in 6 normal monkeys before and after treatment.

**Results:** In normal monkeys, a single dose of WIN 55212-2 reduced intraocular pressure for 4, 5, or 6 hours, with a maximum reduction of 1.4±0.4 (mean±SEM) mm Hg, 2.9±0.4 mm Hg, and 3.4±0.6 mm Hg following the 0.07%, 0.2%, and 0.5% concentrations, respectively (P=.08). In 8 glaucomatous monkey eyes, the ocular hypotensive effect was maintained for 5 days with twice-daily administration of 0.5% WIN 55212-2. Outflow facility was unchanged (P=.34) and aqueous humor flow was decreased by 18% (P=.04) in the treated eyes compared with vehicle-treated contralateral control eyes in normal monkeys.

**Conclusions:** WIN 55212-2, a cannabinoid agonist at the CB1 receptor, reduces intraocular pressure in both normal and glaucomatous monkey eyes. A decrease of aqueous flow appears to account for the intraocular pressure reduction in normal monkey eyes.

**Clinical Relevance:** Cannabinoid agonists at the CB1 receptor, a new class of antiglaucoma agents that is different from currently used clinical drugs, may have clinical potential.

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**Methods**

We used 6 normotensive monkeys and 8 monkeys in which glaucoma had been induced unilaterally by repeated argon or diode laser photocoagulation of the midtrabecular meshwork for 360°. The Macaca cynomolgus monkeys were adult females that weighed from 3 to 5 kg. On each day of the study, IOP was measured with a calibrated pneumotonometer (Model 30 Classic; Mentor Inc, Norwell, Mass) before drug administration, then hourly until 6 hours after drug administration. The monkeys were sedated with intramuscular ket-
amino hydrochloride (1-5 mg/kg), and 1 drop of 0.5% proparacaaine hydrochloride was administered topically 5 minutes before IOP and outflow facility measurements.

WIN 55212-2 (Sigma-Aldrich Corp, St Louis, Mo) solution was freshly prepared by dissolving in a vehicle of 45% 2-hydroxypropyl-β-cyclodextrin (Sigma-Aldrich). Single-dose testing was performed using concentrations of 0.07%, 0.2%, and 0.5% WIN 55212-2 in 5 normal monkeys. In normal monkeys, 50 µL (2 × 25 µL) of WIN 55212-2 was applied to the right eye, and an equal volume of the vehicle was applied to the left eye. A crossover study was then performed in which the same volume of vehicle was instilled in the contralateral control eye at 8:30AM. Flow rates were measured at the same time as on the baseline day, beginning 1 hour after drug application. The washout period between each test on the same animal was at least 1 week.

The 2-tailed paired t test was used for statistical analysis before and after single-dose treatment, and the Bonferroni t test was used for analysis of the multiple-dose study. P < .05 was considered statistically significant. Data were calculated as the mean ± SEM. All experiments complied with the Association for Research in Vision and Ophthalmology Resolution on the Use of Animals in Research and were approved by the Mount Sinai School of Medicine (New York, NY) Institutional Animal Care and Utilization Committee.

**RESULTS**

In 5 normal monkeys (n=10 eyes), unilateral application of WIN 55212-2 significantly (P < .05) reduced IOP for up to 4, 6, and 7 hours following the 0.07%, 0.2%, and 0.5% concentrations, respectively (P = .02). The maximum differences in IOP occurred at 3 hours after drug application and were 1.4 ± 0.4 mm Hg (8% less than in vehicle-treated eyes) with the 0.07% concentration, 2.9 ± 0.4 mm Hg (18% less) with the 0.2% concentration, and 3.4 ± 0.6 mm Hg (19% less) with the 0.5% concentration compared with the vehicle-treated contralateral control eyes (Figure 1). In the 8 glaucomatous monkeys, 0.5% WIN 55212-2 significantly (P < .05) reduced IOP up to 3.5 ± 1.2 mm Hg (10%) on day 1, 5.9 ± 1.3 mm Hg (17%) on day 3, and 8.3 ± 1.7 mm Hg (24%) on day 5 compared with the baseline measurements (P = .02) (Figure 2). A significant reduction in IOP was observed at 2 hours and 6 hours after dosing on day 1 and for at least 6 hours after dosing on days 3 and 5. The magnitude and duration of the ocular hypotensive effect were enhanced with twice-daily administration for 5 days. Significant differences in IOP were not observed when the baseline and vehicle-treated days were compared during the 5 days of treatment. Tachyphylaxis and anterior segment inflammation were not observed during the multiple-dose study.

Three hours after unilateral application of 50 µL of 0.5% WIN 55212-2 to 6 normal monkeys, outflow facility was unchanged (P = .34). The IOP measured topographically at 3 hours after 0.5% WIN 55212-2 administration in treated eyes was reduced by 11% when...
compared with either baseline values or contralateral control eyes (P < .001). For 4 hours following a single dose of 0.5% WIN 55212-2 to the treated eyes of 6 normal monkeys, aqueous humor flow rates were significantly (P < .05) reduced by 18% compared with contralateral vehicle-treated eyes (P = .04) (Table).

**COMMENT**

Cannabinoid agonists can be classified as classic cannabinoids, bicyclic cannabinoids, fatty acid amides and esters, and aminoalkylindoles. WIN 55212-2 is a synthetic aminoalkylindole that selectively binds to the CB1 cannabinoid receptor.

The results of this study show that WIN 55212-2 significantly reduces IOP both in normal and glaucomatous monkey eyes. The reduction in IOP appears to be dose-dependent. Single-dose testing in normal monkeys shows that the 0.2% dose of WIN 55212-2 produces a greater magnitude (19% vs 8%) and longer duration (5 hours vs 4 hours) of IOP reduction than the 0.07% concentration. Increasing the concentration to 0.5% produces a further small IOP reduction, indicating that 0.5% appears to be near the top of the dose-response curve. Five-day, twice-daily treatment with 0.5% WIN 55212-2 in 8 glaucomatous monkey eyes produces a sustained reduction in IOP and an increase in the ocular hypotensive effect with repeated dosing without tachyphylaxis or anterior segment inflammation. These IOP data are consistent with previous animal and human studies.

The mechanism by which WIN 55212-2 lowers IOP is not yet known. Cannabinoids may act directly as vasodilators of the efferent blood vessels of the anterior uvea, improving aqueous outflow. WIN 55212-2 seems to act through CB1 receptors in the eye to mediate the IOP response. Recent studies have demonstrated the presence of functional CB1 receptors in the ciliary processes and trabecular meshwork from animal and human tissues. This suggests that the IOP-lowering effects of cannabinoids could result from activation of CB1 receptors in the ciliary processes (decrease aqueous humor production), in the trabecular meshwork (increase outflow facility), or both. In addition, another recent study provides evidence that undegraded arachidonylethanolamide, an endogenous cannabinoid, lowers IOP in normotensive rabbits via a CB1 receptor mechanism.

Our study demonstrates that topical application of WIN 55212-2 does not alter the outflow facility but does cause a significant reduction in aqueous humor flow of up to 18% in treated eyes compared with contralateral control eyes. We note that there is no statistical difference in aqueous humor flow between treated eyes and their baseline flow measurements, which may reflect the small sample size and the small IOP reductions in normal monkeys.

The role of the cannabinoid receptor in the modulation of aqueous humor dynamics is not completely understood. In the present study, the 18% decrease in aqueous humor production appears to be insufficient to account for the total reduction of IOP that WIN 55212-2 produced. In comparison, 2% dorzolamide, a topical carbonic anhydrase inhibitor, reduced the aqueous humor flow rate by 38%, and 0.5% oxymetazoline, a selective a2-adrenergic agonist, reduced the aqueous humor flow rate by 37% in normal monkey eyes compared with vehicle-treated control eyes. This suggests that additional mechanisms may also be involved in the IOP-lowering effect of WIN 55212-2.

This study is the first to demonstrate that the application of a single dose or multiple doses of WIN 55212-2, a specific CB1 cannabinoid receptor agonist, reduces IOP in normal and glaucomatous monkey eyes and does so in part by decreasing aqueous humor flow. CB1 cannabinoid receptor agonists are a new class of antiglaucoma agents that may have clinical potential.

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Corresponding author and reprints: Rong-Fang Wang, MD, Box 1183, Mount Sinai School of Medicine, One Gustav A.

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**Table**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Intraocular Pressure, mm Hg</th>
<th>Outflow Facility, µL/min per mm Hg</th>
<th>Aqueous Humor Flow, µL/min</th>
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<tbody>
<tr>
<td>WIN 55212-2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>17.5 ± 2.0</td>
<td>0.58 ± 0.03</td>
<td>1.83 ± 0.14</td>
</tr>
<tr>
<td>Treated</td>
<td>15.5 ± 1.5†</td>
<td>0.65 ± 0.08</td>
<td>1.60 ± 0.14†</td>
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<tr>
<td>Vehicle</td>
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<tr>
<td>Baseline</td>
<td>17.0 ± 2.1</td>
<td>0.55 ± 0.07</td>
<td>1.77 ± 0.15</td>
</tr>
<tr>
<td>Treated</td>
<td>17.5 ± 1.6</td>
<td>0.61 ± 0.04</td>
<td>1.96 ± 0.17</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SEM.
†Significant difference in aqueous flow rates compared with baseline or contralateral vehicle-treated eyes (2-tailed paired t test; P < .001).
‡Significant difference in aqueous flow rates compared with contralateral vehicle-treated eyes (2-tailed paired t test; P = .04).
REFERENCES


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