Effect of WIN 55212-2, a Cannabinoid Receptor Agonist, on Aqueous Humor Dynamics in Monkeys

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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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THE INTRAOCULAR pressure (IOP) lowering effect of marijuana smoking was first reported in the 1970s. Since then, many studies have demonstrated the ocular hypotensive effects of different classes of cannabinoids—either endogenous or exogenous, natural or synthetic—in a variety of animals, including humans.

Aminoalkylindoles are a new class of cannabimimetics with a structure entirely different from that of other natural cannabinoids. WIN 55212-2, (R)-(+)-[2,3-dihydro-5-methyl-3-[[(morpholino)methyl]pyrrolo-[1,2,3-de]-1,4-benzoxazinyl(1-naphthalenyl)methanone mesylate, a prototypic aminoalkylindole, has been shown to reduce IOP in rabbits and patients with glaucoma. The mechanism by which WIN 55212-2 reduces IOP is unclear. Recent studies have shown that WIN 55212-2 binds to specific CB1 cannabinoid receptors, suggesting that the IOP-lowering effect of WIN 55212-2 involves this subtype of cannabinoid receptors.

The purpose of the present study is to evaluate the effects of WIN 55212-2 on IOP following single-dose and multiple-dose applications in normal and glaucomatous monkey eyes and to determine the mechanism by which WIN 55212-2 alters IOP in normal monkeys.

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 pneumatometer (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-
Aqueous humor flow measurements were performed with a scanning computerized fluorophotometer (Fluorotron; Coherent Inc, Palo Alto, Calif) in 6 normal monkeys. Fluorescein was iontophoresed into the central corneas of both eyes (with an electrode of 10% fluorescein in 2% agar gel) for 7 minutes at 4 PM on the day before aqueous flow measurements were taken. Baseline aqueous humor flow rates were measured hourly for 4 hours beginning at 9:30 AM. A 50 µL (2 × 25 µL) drop of 0.5% WIN 55212-2 was applied to one eye of each monkey and the same volume of vehicle was instilled in the contralateral control eye at 8:30 AM. Flow rates were measured at the same times as on the baseline day, beginning 1 hour after drug administration. 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, 5, and 6 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 chamber 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 tonographically at 3 hours after 0.5% WIN 55212-2 administration in treated eyes was reduced by 11% when...
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 CB\textsubscript{1} cannabinoid receptor.\textsuperscript{9,13-16} 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\textsuperscript{11} and human\textsuperscript{13} 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.\textsuperscript{13} WIN 55212-2 seems to act through CB\textsubscript{1} receptors in the eye to mediate the IOP response.\textsuperscript{9-11,16} Recent studies have demonstrated the presence of functional CB\textsubscript{1} receptors in the ciliary processes and trabecular meshwork from animal and human tissues.\textsuperscript{16,18,19} This suggests that the IOP-lowering effects of cannabinoids could result from activation of CB\textsubscript{1} 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 CB\textsubscript{1} receptor mechanism.\textsuperscript{20}

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 \(\alpha_2\)-adrenergic agonist, reduced the aqueous humor flow rate by 37% in normal monkey eyes compared with vehicle-treated control eyes.\textsuperscript{21,22} 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 CB\textsubscript{1} cannabinoid receptor agonist, reduces IOP in normal and glaucomatous monkey eyes and does so in part by decreasing aqueous humor flow. CB\textsubscript{1} cannabinoid receptor agonists are a new class of antiglaucoma agents that may have clinical potential.

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