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.1 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,2-11 including humans.1,12-15

Aminoalkylindoles are a new class of cannabimimetics with a structure entirely different from that of other natural cannabinoids. WIN 55212-2, (R)-(+)\[2,3-dihydro-5-methyl-3\-(morpholinyl) methyl]pyrrolo\-[1,2,3-de]-1,4-benzoxazinyl\]-1-(naphthalenyl) methanone mesylate, a prototypic aminoalkylindole, has been shown to reduce IOP in rabbits11 and patients with glaucoma.15 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.11,15,16

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 photoagulation of the midtrabecular meshwork for 360°.17 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-
and one vehicle-treated day, 50 µL of 0.5% WIN 55212-2 was applied to the glaucomatous eye twice daily at 9:30 AM and 3:30 PM for 5 consecutive days. Diurnal IOP measurements were taken on days 1, 3, and 5 after morning dosing.

Outflow facility was measured with an electronic indentation tonograph (EDT-130; Alcon Laboratories, Ft Worth, Tex) prior to drug administration and was repeated 3 hours after unilateral application of 50 µL (2 × 25 µL) of 0.5% WIN 55212-2 in 6 normal monkeys.

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 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, 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 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 tonographically at 3 hours after 0.5% WIN 55212-2 administration in treated eyes was reduced by 11% when
flow rate by 38%, and 0.5% oxymetazoline, a selective α2-adrenergic agonist, reduced the aqueous humor flow rate by 37% in normal monkey eyes compared with vehicle-treated control eyes.21,22 This suggests that additional mechanisms may also be involved in the IOP-lowering effect of WIN 55212-2.

This study was 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 anti-glaucoma agents that may have clinical potential.

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