Objective: To determine the efficacy of 0.5-mg and 0.1-mg sustained-release fluocinolone acetonide intravitreal implants to inhibit ocular inflammation in a rabbit model of severe uveitis.

Methods: The in vitro pharmacokinetic profile of both the 0.5-mg and 0.1-mg sustained-release fluocinolone intravitreal implants was determined during a 10-day period. A sustained-release fluocinolone acetonide intravitreal implant with a release rate of either 0.5 µg/d (n=16) or 0.1 µg/d (n=16) was implanted into the vitreous cavity of the right eye in albino rabbits after a subcutaneous injection of tuberculin antigen. Control animals (n=14) received empty devices. Uveitis was induced with an intravitreal tuberculin antigen injection. A masked observer graded anterior chamber flare, anterior chamber cells, vitreous opacity, and inflammation on histologic sections.

Results: In vitro, the drug was released from both devices in a linear manner. In vivo, treated eyes were significantly less inflamed than untreated eyes (P<.02). Inflammation was suppressed to a greater degree with the 0.5-µg/d implant compared with the 0.1-µg/d implant.

Conclusion: Sustained-release fluocinolone intravitreal implants suppress ocular inflammation in a rabbit model of severe uveitis.

Clinical Relevance: The efficacy demonstrated with the 0.1-µg/d implant provides the rationale for future human studies with lower-release-rate implants than are currently used in noninfectious uveitis clinical trials.

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UVEITIS IS A CHRONIC DISEASE that often requires long-term medical treatment. Vision-threatening complications may occur because of inadequate suppression of inflammation or as an adverse effect of the treatment itself. Medications to treat uveitis can be delivered by topical drops, local injections (subconjunctival, posterior sub-Tenon, or intravitreal), or systemically. Each of these administration routes has drawbacks and potential complications.1-10

We have previously investigated intracocular sustained delivery systems to treat severe uveitis and found that intravitreal sustained-release devices that contain dexamethasone and cyclosporine effectively control inflammation without producing systemic adverse effects.11-13 Fluocinolone acetonide, a synthetic corticosteroid with low aqueous solubility, has been incorporated into an implant system that can deliver the drug at a constant release rate for a prolonged period (eg, a device with a 2-mg drug core and a release rate of 2 µg/d of fluocinolone acetonide has a predicted lifespan of approximately 3 years).16

In a dose-masked multicenter study of 278 patients treated with either a 2.0-µg/d or 0.5-µg/d release-rate fluocinolone acetonide intravitreal implant, there was a significant reduction in recurrent inflammation in the implanted eye and a reduction in oral and topical anti-inflammatory medication use. This effect was maintained during the 24-month follow-up period.17

Currently, the optimal fluocinolone intravitreal implant release rate to treat uveitis is unknown. We hypothesized that sustained delivery of fluocinolone targeting the posterior segment would control inflammation at release rates that are lower than those currently used in clinical trials. To test this hypothesis, we studied the efficacy of 0.5-mg and 0.1-mg sustained-release fluocinolone acetonide intravitreal implants in a rabbit model of severe uveitis.
ous described. Briefly, drug powder with pharmaceutical excipients was compressed into 1.5-mm tablets. Pellets were then coated with a polyvinyl alcohol and silicone laminate and affixed to a polyvinyl alcohol suture strut. The assembly was heat treated at 135°C for 5 hours to change the polyvinyl alcohol crystalline structure and to control the drug delivery rate. The devices were then sterilized with γ-irradiation before implantation. Release occurred through a diffusion port in the coating.

IN VITRO PHARMACOKINETICS

For both the 0.5-µg/d and 0.1-µg/d implants, drug release was determined by placing 8 devices into separate microcentrifuge tubes, each containing 1 mL of 0.1M phosphate-buffered saline (pH 7.4; 37°C). At approximately 24-hour intervals during a 10-day period, the entire buffer was removed for analysis by reverse-phase high-performance liquid chromatography and then replaced. The high-performance liquid chromatography analysis was performed using a fully automated system (Hitachi, San Jose, Calif) with a reverse phase and guard columns (C-18; Axion Chromatography, Moorpark, Calif). The mobile phase was a 40:60 mixture of acetonitrile and 0.02% sodium acetate (pH 4.0) with detection at 238 nm. For each device, the release rate was calculated from graphs of cumulative drug release vs time. The mean release rate was the mean of the individual device release rates in each batch.

PREIMMUNIZATION WITH ANTIGEN

Experiments were conducted in accordance with the guidelines set forth by the Association for Research in Vision and Ophthalmology’s Statement for the Use of Animals in Ophthalmic and Vision Research. The study was approved by the Duke University Institutional Animal Care and Use Committee. A severe, predominantly T-cell–mediated uveitis model was created according to a previously published protocol. Forty-six New Zealand white rabbits, each weighing 1.5 to 2.0 kg, were injected subcutaneously with 10 mg of Mycobacterium tuberculosis H37Ra antigen (Difco, Detroit, Mich) suspended in 0.5 mL of mineral oil. A firm, discrete nodule was identified at the injection site approximately 7 to 10 days after antigen injection, confirming the efficacy of immunization.

DEVICE IMPLANTATION

Fourteen days after the subcutaneous tuberculin antigen injection, sustained-release fluocinolone acetonide intravitreal implants (either 0.1 µg/d [n = 16] or 0.5 µg/d [n = 16]) were surgically implanted into the right eyes of 32 animals according to a previously described method. The devices were inserted into the vitreous cavity through a sclerotomy 1.5 mm posterior to the limbus and suspended at the sclerotomy site by an 8-0 nylon suture. The sclerotomy was then closed with 8-0 nylon sutures, and the conjunctival peritomy was closed with 7-0 8-0 nylon suture. The anterior chamber (AC) cells and flare, and vitreous opacity were quantified according to previously reported grading systems.

AQUEOUS PROTEIN MEASUREMENT AND LEUKOCYTE COUNT

Three animals in each group were randomly chosen on day 6 for aqueous protein measurement and leukocyte count. Animals were anesthetized, and 0.1 mL of aqueous humor was aspirated from the right eye of each rabbit with a heparin-rinsed syringe connected to a 30-gauge needle. The animals were then euthanized. Ten microliters of the aqueous solution was used for cell count by hemocytometer (Hauser Scientific, Horsham, Pa). The remaining aqueous solution was centrifuged at 800 rpm for 5 minutes. The supernatant was removed and set aside for protein measurement. The pellet was resuspended, and 1 drop of this concentrated aqueous sample was placed on a microscope slide and stained with Wright stain for differential cell count. The protein content of the aqueous humor was determined using an assay kit (BioRad, Richmond, Calif) with bovine serum albumin as a standard dilution reference curve according to the manufacturer’s recommendation.

ELECTORETINOGRAPHY

Retinal function was evaluated by scotopic electrorretinography using an EPIC-2000 unit (LKC Technologies, Gaithersburg, Md) with a Ganzfeld flash in 4 animals from each group, randomly selected on day 5 after the intravitreal challenge. Scotopic electrorretinograms were performed on each eye after at least 30 minutes of dark adaptation. The b-wave amplitude ratio of the antigen-challenged eye to the fellow eye was used as an index of retinal function.

HISTOPATHOLOGIC STUDY

Right eyes were enucleated from rabbits in each of the 3 implant groups at day 6 after the intravitreal challenge, and 5-µm paraffin sections were obtained and stained with hematoxylin-eosin. Sections were obtained of anterior (including the cornea, iris, and ciliary body) and posterior segment structures, oriented along the optic nerve and medullary ray, and examined by light microscopy. Sections were presented to 2 masked observers (P.M. and G.J.J.) who were asked to judge whether they were obtained from the 0.1-µg/d– or 0.5-µg/d–treated or control eyes based on the degree of inflammation, using standard reference sections for comparison. In addition, a masked grader (R.T.) counted all infiltrating inflammatory cells in 8 random, noncontiguous fields at ×200 magnification in both the anterior and posterior segment sections. A semilogarithmic grading scale, modified from Verma et al., was used to compare median inflammatory cell infiltrate among the 3 groups. The grades were as follows: grade 0, no cells per field; grade 1, 1 to 10 cells per field; grade 2, 11 to 30 cells per field; grade 3, 31 to 100 cells per field; and grade 4, 101 to 300 cells per field.
The median value for each measure for each implant group at each day of the study was computed. For each measure of inflammation, including aqueous protein concentration and leukocyte count, median values were compared among groups at each day of the study using the Kruskal-Wallis test. Area under the curve (AUC) comparisons between the control and treatment groups were performed to assess trends in each measure during the entire course of the study. Differences among 2 groups were considered statistically significant if \( P < .05 \). Pairwise comparisons were made between groups using the Wilcoxon rank sum test. For each measure, comparison among and between groups at each day were of interest in their own right. Thus, adjustments for multiple comparisons were made separately for each measure and day. The Bonferroni correction was used. Since 3 tests were performed, results were considered statistically significant if \( P < .017 \ (0.05/3) \). Data were analyzed using SAS statistical software, version 8.1 (SAS Institute Inc, Cary, NC).

IN VITRO PHARMACOKINETIC DATA

Fluocinolone acetonide release for both the 0.5-µg/d and 0.1-µg/d implants was linear during the 10-day sampling period, and no evidence of fluocinolone oxidation or degradation was observed by high-performance liquid chromatography (Figure 1). Because of the extremely low release rate of the 0.1-µg/d implant, the release rate standard deviation was relatively high compared with the 0.5-µg/d implant.

CLINICAL OBSERVATIONS

Device implantation was performed in all animals without complication. Specifically, no surface bleeding, vitreous hemorrhage, lens touch during implantation, dislocation of the implant, or retinal detachment occurred.

The AC cells, AC flare, and vitreous opacity clinical grades after intravitreal tuberculosis antigen injection are shown in Figure 2A-C, respectively. Clinical signs of ocular inflammation appeared on day 1 after intravitreal challenge; all eyes had AC flare and vitreous opacity on day 1 and AC cells by day 4. Intraocular inflammation peaked in all 3 groups between days 1 and 4 after intravitreal challenge. In the control group, AC cells, AC flare, and vitreous opacity peaked on day 3 and persisted through day 21. In the 0.1-µg/d group, AC cells and AC flare peaked on day 3 and remained constant through day 6. In the 0.5-µg/d group, AC cells peaked on day 4 and AC flare peaked on day 1 and then gradually declined to undetectable levels. In both treatment groups, vitreous opacity peaked on day 1 but to a slightly lower degree in the 0.5-µg/d group, and vitreous opacity was undetectable in either group by day 5 after intravitreal challenge.

Treated eyes in both implant groups had significantly less anterior segment inflammation than control eyes. Overall, there was a significant difference among all 3 groups by AUC analysis for AC cells \( (P = .015) \) and AC flare \( (P = .008) \). The AC cells (Figure 2A) were significantly reduced by pairwise comparison in all treated eyes on days 4 through 9 \( (P = .004) \), and AC flare (Figure 2B) was significantly reduced on day 1 and then days 3 through 9 \( (P = .002) \). Eyes in the 0.5-µg/d group, compared with controls, had a significant reduction in AC cells on days 4 through 9 \( (P = .006) \) and in AC flare on day 1 and days 3 through 9 \( (P = .006) \). Similarly, eyes in the 0.1-µg/d group, compared with controls, had a significant reduction in AC cells on days 4 and 9 \( (P = .016) \) and in flare on days 3 to 5, 7, and 9 \( (P = .01) \). By AUC analysis, there was significantly less AC flare in eyes with the 0.5-µg/d implant compared with the 0.1-µg/d implants \( (P = .02) \) and specifically on days 1, 3, 6, and 7 \( (P = .03) \). There was no significant difference in AC cells by AUC analysis \( (P = .52) \) between the 2 treatment groups.

Treated eyes in both implant groups had significantly less vitreous inflammation, measured by vitreous opacity, than control eyes. Overall, by AUC analysis, there was a significant difference in vitreous opacity among all 3 groups \( (P = .003) \). Vitreous opacity (Figure 2C) was significantly reduced in treated eyes (both implant groups) compared with untreated eyes on days 2 to 9 \( (P = .002) \). Pairwise comparisons for days 2 through 9 showed differences between each treatment group and the control group \( (P < .002) \) for all tests. By AUC analysis, there was significantly less vitreous opacity in eyes with the 0.5-µg/d implant compared with the 0.1-µg/d implants \( (P = .01) \), but significant differences in pairwise comparisons were seen only on days 1 \( (P = .027) \), 4 \( (P = .045) \), and 6 \( (P = .041) \). Clinically, in untreated eyes, the vitreous was diffusely opaque with striking condensed vitreous opacities often noted in the inferior vitreous cavity overlying the peripheral retina. These opacities were not observed in either fluocinolone implant group and clinically resembled snowballs and snowbanks observed in patients with pars planitis.

Corneal neovascularization was mild in all groups and stabilized after day 4. No differences were seen between the groups. Iris congestion was significantly reduced in treatment groups compared with controls from days 3 through 7 and on day 9 \( (P = .02) \) on each of these days). This reduction was more pronounced, compared with controls, in the 0.5-µg/d group on these days \( (P = .01) \) but only on day 9 in the 0.1-µg/d group.
ELECTRORETINOGRAPHY

The b-wave amplitude ratios for treated and untreated eyes paralleled the clinical observations (Figure 3). The b-wave ratio was moderately depressed in untreated eyes. Both high- and low-release-rate groups showed a mild depression in b-wave ratio. Differences in b-wave ratio between treated and untreated groups were not statistically significant.

AQUEOUS PROTEIN CONCENTRATION AND LEUKOCYTE COUNT

The aqueous protein concentration and white blood cell count paralleled the clinical assessment of AC cells and flare. Protein concentration and white blood cell count were highest in untreated eyes. Treated eyes from each implant group had a marked, although nonsignificant, reduction in protein concentration and white blood cell count compared with untreated eyes (Figure 4A and B). The 0.1-µg/d group showed marginally higher, although not statistically significant, levels of protein ($P=0.38$) and white blood cells ($P=0.24$) compared with the 0.5-µg/d group.

HISTOPATHOLOGIC ANALYSIS

Representative histopathologic sections of the control and 2 fluocinolone implants are shown in Figure 5. At each point, masked observers were able to correctly identify whether the eye was from the 0.5-µg/d, 0.1-µg/d, or control group based on the severity of the histologic manifestations of intraocular inflammation (Figure 5). The median anterior segment inflammatory grade was 3, 1, and 0 for the control, 0.1-µg/d, and 0.5-µg/d groups, respectively. The median posterior segment inflammatory grade was 3, 0, and 0 for the control, 0.1-µg/d, and 0.5-µg/d groups, respectively.

COMMENT

In this study, sustained-release intravitreal implants that contained fluocinolone acetonide at release rates of 0.5 µg/d and 0.1 µg/d reduced ocular inflammation in a rabbit model of severe uveitis as determined by clinical examination, aqueous cell and protein quantification, electroretinography, and histologic examination. Overall, ocular inflammation was suppressed earlier during the study and to a greater degree by the 0.5-µg/d (higher) release-rate implant compared with the 0.1-µg/d (lower) release-rate implant. However, the lower-release-rate device still effectively inhibited ocular inflammatory signs compared with control eyes.

In the present study, ocular inflammation was inhibited with both devices to a greater degree in the posterior...
segment of the eye (eg, vitreous opacity) than in the AC (eg, aqueous cells and flare). Although both release-rate implants suppressed vitreous opacity, the 0.5-µg/d–treated eyes demonstrated greater inhibition of anterior segment inflammation compared with the 0.1-µg/d–treated eyes. Driot et al reported up to an 81-fold higher fluocinolone concentration in the New Zealand Black Satin Cross rabbit vitreous cavity, compared with aqueous humor, 6 months after implantation of a 0.5-mg sustained-release fluocinolone intravitreal implant. The data from our study suggest that in the 0.5-µg/d–treated eyes, despite the difference in aqueous and vitreous fluocinolone concentration, drug levels were sufficient to suppress both anterior and posterior segment inflammation. The fluocinolone acetonide release rate for the 0.1-µg/d implant was lower than that of the 0.5-µg/d implant (Figure 1) and would likely produce lower aqueous and vitreous fluocinolone levels. Accordingly, a proportionate difference in aqueous and vitreous humor concentrations in the 0.1-µg/d–implanted eyes may explain why in the current study there was less suppression of aqueous cells and flare in the 0.1-µg/d– compared with the 0.5-µg/d–treated eyes. Regardless, the fluocinolone acetonide vitreous concentration in the 0.1-µg/d–treated eyes was sufficient to suppress posterior segment inflammation.

Intravitreal sustained-release devices have also been formulated with other corticosteroids. Cheng et al demonstrated, by the same criteria used in this study, that eyes with a dexamethasone implant had significantly less intraocular inflammation compared with controls. The effect of the dexamethasone device on ocular inflammation, measured by AC cells and AC flare, was marginally better than our current data from the 0.1-µg/d fluocinolone acetonide implant and nearly identical to that from the 0.5-µg/d fluocinolone acetonide implant. Vitreous opacity, however, was not detectable in either fluocinolone implant group by day 5 compared with low-grade persistent opacity in the dexamethasone-treated eyes. The uveitis model used by Cheng and colleagues differed from that used in the present study; those authors used a crude preparation of the tuberculin antigen that retained cell wall components and resulted in a nonspecific inflammatory cell reaction, compared with a more pure T-cell–mediated reaction in the current model. These model differences may account for the differences in vitreous opacity between the studies. It is unclear whether these vitreous opacity differences would be clinically significant in humans.

Our study has some limitations. First, we assessed drug efficacy in a short-duration, small-animal uveitis model using a delivery device intended for long-term drug delivery in humans. The rabbit panuveitis model mimics the clinical picture seen in humans with severe panuveitis who have rapid escalation followed by continuing low-grade inflammation. By implanting the fluocinolone device after sensitization by subcutaneous M tuberculosis antigen but before intravitreal antigen injection, we have recreated the clinical scenario whereby sustained-release fluocinolone intravitreal implants would be used in patients with a history of recurrent uveitis but without active inflammation to provide a steady-state drug concentration within the target eye. Then, by injecting the intravitreal antigen, drug efficacy is being assessed in a setting similar to a severe uveitis flare-up. To determine the long-term anti-inflammatory effect of fluocinolone implants, it would be necessary to give repeated intravitreal injections, a method we avoided to minimize animal discomfort caused by uncontrolled inflammation in eyes not receiving a fluocinolone implant. Second, the rabbit model does not allow for accurate determination of corticosteroid-induced intraocular pressure elevation. Third, although no lenticular opacities were attributed to the fluocinolone or implant itself, the long-term effect of the 2 fluocinolone devices on cataract formation has yet to be determined. In previous reports, higher-release-rate fluocinolone intravitreal implants did not cause cataract formation during the course of 1 year in the rabbit model. Regardless, corticosteroid-induced cataract rates in rabbits may differ from those seen in humans.

This study was designed to assess the efficacies of both a 0.5-µg/d and 0.1-µg/d sustained-release device in a T-cell–predominant uveitis model. The model pro-

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Figure 3. Ratio of b-wave amplitudes (antigen-challenged eye to fellow eye) at day 6 after intravitreal challenge. Values represent means ± SD (error bars).

Figure 4. Quantitative measurement of aqueous leukocytes (A) and aqueous protein (B) at day 6 after intravitreal challenge. Values represent means ± SD (error bars). P values overlying 0.1- and 0.5-µg/d data represent 2-tailed comparison with the untreated group.
duced a high degree of inflammation, which was clearly inhibited by both implants. Eyes treated with a 0.5-µg/d implant demonstrated a greater anti-inflammatory effect than eyes treated with the 0.1-µg/d implant. However, at the peak of the inflammatory reaction produced by the model (days 3–5), there was no significant difference in efficacy between the 2 devices by any parameter used in the study. These results provide the rationale for use of the 0.5-µg/d fluocinolone acetonide intravitreal implant in ongoing clinical trials. Further clinical studies are needed to determine whether implants that provide lower release rates, such as the 0.1-µg/d implant used in our study, can provide therapeutic efficacy while decreasing drug-associated adverse effects.

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Author Contributions: Drs Mruthyunjaya and Jaffe had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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