Safety and Pharmacokinetics of an Intraocular Fluocinolone Acetonide Sustained Delivery Device

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PURPOSE. To determine the safety and pharmacokinetics of an intraocular fluocinolone acetonide sustained drug delivery device.

METHODS. Nonbiodegradable drug delivery devices containing 2 or 15 mg of a synthetic corticosteroid, fluocinolone acetonide, were constructed. The long-term in vitro release rates of these devices were determined in protein-free buffer or buffer containing 50% plasma protein. Fifteen-milligram devices were also implanted into the vitreous cavities of rabbit eyes. Intravitreal drug levels, the amount of drug remaining in explanted devices, and the release rate of explanted devices were determined over a 1-year time period. Drug toxicity was assessed over this same time period by slit lamp examination, indirect ophthalmoscopy, electroretinography, and histologic examination.

RESULTS. The drug release rates for the 2-mg device, 1.9 ± 0.25 μg/d, and for the 15-mg device, 2.2 ± 0.6 μg/d, remained linear over the 6-month and 45-day testing period, respectively. The release rate increased by approximately 20% when devices were transferred from protein-free buffer to buffer that contained protein (P < 0.0001). Vitreous levels remained fairly constant (0.10–0.21 μg/ml) over a 1-year period. No drug was present in the aqueous humor during this time period. Based on the device release rates, the predicted life span of the 2- and 15-mg devices are 2.7 and 18.6 years, respectively. There was no evidence of drug toxicity by clinical examination, electroretinography, or histologic examination.

CONCLUSIONS. It is feasible to construct a nontoxic fluocinolone acetonide drug delivery device that reproducibly releases fluocinolone acetonide in a linear manner over an extended period. These devices show great promise in the treatment of ocular diseases such as uveitis, which are often managed with chronic corticosteroid therapy. (Invest Ophthalmol Vis Sci. 2000;41:3569–3575)

Uveitis is often a chronic disease that requires long-term medical therapy. Corticosteroids given topically, systemically, or as a periocular injection are the mainstays of uveitis treatment.1 However, each of these delivery methods has potential drawbacks. Topical and systemic treatment require rigorous patient compliance over an extended period to effectively deliver these medications. Topical corticosteroids usually do not control posterior segment disease. Oral corticosteroids are associated with numerous systemic side effects that may be treatment limiting and in some cases may be life-threatening.2,3 Periocular injections may cause periocular fibrosis, globe perforation, and ptosis. With all three delivery methods, it is often difficult to achieve adequate ocular drug levels without unacceptable side effects.

To overcome the disadvantages of topical, systemic, and periocular corticosteroid delivery, we have investigated the safety and efficacy of intraocular corticosteroid sustained delivery devices. We have shown that a dexamethasone delivery device is safe and effective in the treatment of severe experimental panuveitis.4 Recently, we implanted a dexamethasone sustained drug delivery device in the eye of a patient with severe panuveitis. The dexamethasone device controlled the intraocular inflammation for 10 months. However, after 10 months, the intraocular inflammation recurred as the device and tissues were depleted of dexamethasone (Jaffe, unpublished results, 1996).

Ideally a corticosteroid delivery device would provide therapeutic drug levels over the duration of the patient's disease. Fluocinolone acetonide is a synthetic corticosteroid with low solubility in aqueous solution. The solubility of fluocinolone acetonide is 1/24 that of dexamethasone, which itself is relatively insoluble. Theoretically, this low solubility should allow very extended drug release from a delivery device without the need for an excessively bulky polymer system. We have undertaken the present study to determine the feasibility of constructing a fluocinolone acetonide device, to test the hypothesis that this device will provide sustained drug delivery in vitro and in vivo, and to evaluate the safety of this device in the rabbit eye.

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**Materials and Methods**

**Device Construction**

A 2- or 15-mg pure fluocinolone acetonide (Spectrum Quality Products, Inc., Gardena, CA) drug core was compressed into a pellet with a customized pellet press (Parr Instruments, Moline, IL). The 2-mg pellet was compressed into a short cylinder, and the 15-mg pellet was compressed into an elongated cylinder. The pellets were affixed to polyvinyl alcohol suture struts and then coated in silicone to form a polyvinyl alcohol and silicone laminate. The assembly was heat-treated at 135°C for 5 hours to change the polyvinyl alcohol crystalline structure and to further control drug release rate. Because the silicone is impermeable to fluocinolone acetonide, drug is released across the polyvinyl alcohol diffusion port in the polyvinyl alcohol/silicone laminate.

**In Vitro Pharmacokinetics**

Drug release was determined by placing 10 devices containing 15 mg of fluocinolone acetonide or 12 devices containing 2 mg fluocinolone acetonide into separate microcentrifuge tubes each containing 1.0 ml of 0.1 M phosphate-buffered saline (PBS; pH 7.4, 37°C). At varying intervals, the tubes were vortexed, and the entire supernatant was removed for analysis by reverse-phase high-pressure liquid chromatography (HPLC) as described below. One milliliter of fresh buffer was then added, and the determination continued for up to 168 days. It has been previously shown that plasma proteins can change the release rate of sustained drug delivery devices. Accordingly, additional experiments were conducted to determine whether the fluocinolone acetonide release rate was altered by the presence of human plasma proteins. Eight devices (containing 2 mg fluocinolone acetonide) were placed in microcentrifuge tubes containing 1.0 ml of 0.1 M PBS and assayed every 24 hours for 3 days as described above. PBS was then replaced with 500% human plasma in 0.1 M PBS and assayed every 24 hours for 7 days. Finally, the 50% human plasma in 0.1 M PBS was once again replaced by 0.1 M PBS and assayed every 24 hours for an additional 5 days.

**Device Implantation**

Experiments were conducted in accordance with the guidelines set forth by the ARVO 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. Devices containing 15 mg fluocinolone acetonide were surgically implanted into the right eyes of 14 rabbits according to a previously described method. Briefly, animals were anesthetized with an intramuscular injection of 0.3 ml ketamine hydrochloride (100 mg/ml/kg) and 0.1 ml xylazine hydrochloride (100 mg/ml/kg). A 5-mm peritomy was made in the supertemporal quadrant of the right eye. A 5-mm sclerotomy was created 1 to 2 mm from the limbus. The device was then inserted into the vitreous cavity through the sclerotomy and suspended at the sclerotomy site by a 6-0 dacron suture. The sclerotomy and the peritomy were then closed with 7-0 vicryl sutures. One drop of topical 0.3% gentamicin solution was instilled into the eyes postoperatively for infection prophylaxis. The fellow eyes served as an untreated control group.

**In Vivo Pharmacokinetic Study**

The right eye of rabbits (drug-device–implanted eyes) were used for pharmacokinetic analysis. Fluocinolone acetonide levels also were measured in the fellow, non–device-implanted eye. Anesthesia was obtained with intramuscular injection of ketamine HCl and xylazine as described above at 4, 20, and 54 weeks (four rabbits at each time point). We elected to wait until 4 weeks to obtain the first aqueous and vitreous samples so that pharmacokinetic data would not be confounded by surgical trauma induced by device implantation. Under anesthesia, 100 μl of aqueous was removed from the anterior chamber at the limbus by a 30-gauge needle attached to a 1-ml syringe. The eyes were enucleated and immediately frozen at −80°C. Animals were killed by intracardiac injection of pentobarbital sodium. The tissues were then prepared for assay as described below.

**Drug Level Analysis**

We chose to analyze the pooled vitreous contents, to obtain a measurement of the average fluocinolone acetonide concentration in the vitreous cavity. The vitreous was dissected from the frozen globe as previously described. The fluocinolone acetonide device was then carefully removed. Vitreous and aqueous from each eye were deproteinized in an equal volume of acetonitrile. After removing the protein precipitate, the supernatant was evaporated, and the residue was reconstituted in acetonitrile for HPLC assay. Assays were performed using a fully automated Hitachi (San Jose, CA) HPLC system with a C-18 reverse-phase column with a C-18 guard column (Axxion Chromatography, Moorpark, CA). A 1:1 mixture of acetonitrile and 0.02% sodium acetate (pH 4.0) was used as the mobile phase. Detection was by ultraviolet light (238 nm).

**Pharmacokinetics of Explanted Devices**

Explanted devices obtained from each of 4 eyes at 4, 20, and 54 weeks were rinsed with deionized water and placed in PBS. Aliquots of PBS were assayed for fluocinolone acetonide by HPLC as described above. The initial protocol called for measurement of drug release over a 15-day time period. We elected to test two of the groups over longer periods of time to further confirm the linearity of drug release. Accordingly, fluocinolone acetonide released into PBS was assayed over 15 days for devices obtained at 54 weeks, over 21 days for devices explanted after 4 weeks, and over 27 days for devices removed at 20 weeks. The mean release rate for all three time points together and for each time point separately was calculated. Devices removed at 20 and 54 weeks were then air-dried, the polymer layers were peeled off, and the drug core was weighed to determine the amount of residual fluocinolone acetonide.

**Toxicity Assessment**

**Clinical Evaluation.** The toxicity of the fluocinolone acetonide devices was determined on eyes used for the pharmacokinetic analysis (described above). Slit lamp examination and indirect ophthalmoscopy were performed at baseline, at weekly intervals for the first 8 weeks, and at monthly intervals thereafter. The following clinical features were sought: anterior chamber cells and flare, iris vessel congestion, cataract, vitreous opacity, retinal opacity, retinal detachment, retinal vascular engorgement, and retinal neovascularization.
Electroretinography. Scotopic electroretinography (ERG) was performed on both eyes of each of nine rabbits at baseline and 1, 4, 8, 28, and 54 weeks after device implantation. Electroretinograms were recorded with the use of contact lens electrodes (ERG-Jet; Universo SA, La Chaux-de-Fonds, Switzerland) with a two-channel clinical signal averager (No. 5200; Cadwell, Kennewick, WA) and a Ganzfeld flash unit (VPA-10; Cadwell). Scotopic ERGs, obtained after at least 30 minutes of dark adaptation, were elicited at 0.34 Hz. For each ERG, 20 stimulus presentations were averaged. To minimize the effect of individual and daily variation on the ERG, the ratio of the experimental (right) eye b-wave amplitude to the control (left) eye b-wave amplitude was determined as previously described.4,7 When the amplitude of the experimental and control eyes are equal, the ratio equals 1. A decrease or increase in the ratio reflects a relative decrease or increase in the b-wave amplitude of the experimental eye.

Histopathologic Analysis. Two rabbits used for ERG analysis were killed at 54 weeks for histopathologic analysis. The experimental and control eyes were immediately enucleated, and small cuts were made at the equator of each eye. Eyes were then fixed in 10% formalin solution. Samples were dehydrated with increasing concentrations of ethanol, cleared with xylene, and embedded in paraffin. Sections were obtained from cuts through the whole globe oriented along the optic nerve and medullary ray from a total of four eyes. Five-micrometer sections were stained with hematoxylin and eosin and examined by light microscopy.

Statistical Analysis
A nonpaired two-tailed Student’s t-test was used to compare the fluocinolone acetone release rates in protein-free and plasma protein–containing buffer. An analysis of variance was used to determine differences in release rate for explanted devices. A paired two-tailed Student’s t-test was used to compare the ERG b-wave amplitude ratio before and after device implantation.

RESULTS
In Vitro Pharmacokinetic Data
The release rates of 2- and 15-mg fluocinolone acetone implants were determined over approximately 6 months and 45 days, respectively. For the 2-mg devices, the release rate was consistent among the 12 devices tested and remained linear over the duration of experiment. The mean release rate (±SD) was 1.9 ± 0.25 µg/d (Fig. 1A). For the 15-mg devices, the mean release rate (SD) was 2.2 ± 0.6. This device gave slightly more variable drug release than the 2-mg devices. However, as with the 2-mg device, the release rate was linear over the duration of the experiment (Fig. 1B).

We have previously shown that drug release from a flurbiprofen/acyclovir device is more rapid when the device is placed in medium containing proteins.5 To determine whether plasma proteins similarly influence fluocinolone acetone release, the release profile was determined for implants placed in medium containing proteins, switched to medium containing human plasma proteins, and then switched back to protein-free medium. The mean release rate (±SD) increased by approximately 20%, from 1.7 ± 0.35 to 2.2 ± 0.52 after the devices were placed in protein containing media (P < 0.0001; Fig. 2).

In Vivo Pharmacokinetics
Aqueous and vitreous levels were measured over 54 weeks in fluocinolone acetone device–implanted eyes and fellow eyes.
without a drug device. In drug device–implanted eyes, the mean vitreous levels varied from 0.10 to 0.21 mg/ml over the experiment. The mean levels were not statistically significantly different at the different time points (Fig. 3). Fluocinolone acetonide was not detected at any time point in the aqueous of drug device–implanted eyes or in the aqueous or vitreous of fellow eyes that did not contain a device.

Analysis of Explanted Drug Delivery Devices

Additional experiments were conducted to determine whether surgical implantation of devices altered their release rates and to measure the amount of fluocinolone acetonide remaining in explanted devices. The mean release rate (±SD) from all three time points (4, 20, and 54 weeks) was 3.26 ± 1.41 µg/d (n = 12). The mean release rate (±SD) did not change significantly over the 54-week testing period; the values (n = 4 at each time point) were 2.92 ± 1.9, 3.29 ± 1.49, and 3.57 ± 0.99 at 4, 20, and 54 weeks, respectively (P = 0.85). The average weight of the devices, corrected for the drug released during the in vitro assay period was 13.89 and 12.35 mg at 20 and 54 weeks, respectively (it was not measured at 4 weeks). Assuming a release rate of 3.26 µg/d and correcting for the amount of drug lost over the in vitro assay period, the calculated average amount of fluocinolone acetonide in the devices before implantation would be 14.3 and 13.6 mg for the 20- and 54-week devices, respectively. These calculated values are within 10% of the fluocinolone acetonide (15 mg) used to form the drug core during the initial device construction.

Clinical Examination

On slit lamp examination and indirect ophthalmoscopy, there was no evidence of drug toxicity in the eyes containing the fluocinolone acetonide drug delivery device. Specifically, the cornea remained clear without evidence of neovascularization, the anterior chamber remained quiet, the iris was not congested, there was no evidence of cataract formation, the vitreous remained clear, and the retina appeared normal during the entire follow-up period. Similarly, the fellow eyes remained normal during the follow-up period.

Electroretinography

Electroretinography was performed as an objective measure of retinal function in drug device–implanted eyes. By electroretinography, the dark-adapted b-wave amplitude ratio was initially slightly greater than 1 during the first 3 weeks of the study. Thereafter, levels remained near 1 for more than 1 year after device implantation (Fig. 4). At 28 weeks, the b-wave amplitude ratio was lower than at other time points (approximately 0.75; P < 0.05); however, by 54 weeks, the ratio (0.89) was once again not significantly different from 1 (P = 0.17).

Histopathologic Analysis

The histopathologic appearance of the cornea, iris, retina, choroid, sclera, and optic nerve was normal in fluocinolone acetonide device–implanted eyes and was similar in appearance to the fellow nondevice-implanted eyes. (Fig. 5).

DISCUSSION

In this study we have demonstrated that it is feasible to construct a fluocinolone acetonide drug delivery device that reproducibly releases fluocinolone acetonide in a linear manner over an extended period. Furthermore, the device is nontoxic, as determined by clinical, electroretinographic, and histopathologic analysis.

In vitro, both the 2- and 15-mg fluocinolone acetonide delivery devices produced linear drug release kinetics. These
linear release kinetics are similar to those observed in our previous study of a dexamethasone drug delivery system. In contrast to the dexamethasone device, the fluocinolone acetone device released drug over a much more protracted period. In fact, the release rate of the fluocinolone acetone devices remained essentially constant over the 54-week testing period, whereas linear drug release with the dexamethasone device was only achieved for 12 weeks. Assuming a constant release rate over the duration of the device life span and assuming that the in vitro release rate is similar to the in vivo release rate, we estimate that the 15-mg device could last 18.6 years in the rabbit eye. The 2-mg devices, which were constructed differently than the 15-mg devices and released fluocinolone at a slightly slower rate, are projected to last 2.7 years. The constant, extended release rate achieved with the fluocinolone acetone device would be advantageous in the treatment of chronic diseases such as uveitis, which may run a course over many years.

The release rates determined from the 15-mg devices were somewhat variable; however, release rate reproducibility was improved by the manufacturing process used to produce the 2-mg devices. Recently, using this modified process we have produced devices containing fluocinolone acetone quantities varying from 0.5 to 6 mg (unpublished results, 1999). Taken together, these results indicate that it is possible to construct

![Figure 4](image1.png)  
**Figure 4.** Electroretinographic b-wave amplitude ratio (drug device-implanted eye:fellow control eye) as a function of time (n = 9).

![Figure 5](image2.png)  
**Figure 5.** Histopathologic section from eye with 15-mg fluocinolone acetone device. Hematoxylin and eosin stain. (A) Iris and ciliary body. Original magnification, ×12. (B) Retina in region of medullary ray. Retina is artifactitionally detached from the RPE (RPE not seen in this section). Original magnification, ×40. (C) Higher power view of (B). Original magnification, ×100.
devices that release fluocinolone acetonide in a reproducible manner, for varying time periods, over a range of release rates. The ability to control drug release duration will be useful to tailor specific devices for ocular diseases that have different disease activity duration.

The fluocinolone devices released drug more rapidly into plasma protein containing media, than into media without protein. This effect is caused by the effect of protein on the chemical properties of the fluocinolone acetonide in the dissolution medium and not by a change in the polymers. In eyes with uveitis, there is breakdown of the blood ocular barrier with an associated influx of proteins.5,7–10 We hypothesize that higher drug levels would be necessary to control inflammation during periods of disease activity, when there is more associated blood ocular barrier breakdown (and consequently more protein in the aqueous and vitreous cavities) and lesser amounts of drug would be required to maintain a quiet eye. If correct, this proposal suggests that release kinetics, which are directly related to the protein concentration, would be advantageous in the management of uveitis, which has fluctuating levels of disease activity and therefore varying amounts of aqueous and vitreous protein. In our study, we only tested a single plasma protein concentration. Further experiments would be necessary to conclude that the fluocinolone acetonide device release rate varied directly with different protein concentrations.

In vivo pharmacokinetic data paralleled those obtained in vitro. Relatively constant vitreous levels were measured at each of the three time points tested over 1 year. A relatively small number of time points were sampled for pharmacokinetic analysis (to maximize the number of rabbits available for clinical and electroretinographic examination). Therefore, we cannot exclude the possibility that fluocinolone acetonide levels at time points before the first 4-week time point or between the other time points were different from those actually measured.

Overall, the measured vitreous fluocinolone acetonide levels (0.1–0.2 µg/ml) were lower than those determined in eyes with the dexamethasone delivery device (2.5 µg/ml). The tissue fluocinolone acetonide levels produced by the delivery device are unknown. We hypothesize that the low vitreous levels measured at steady state, given fluocinolone acetonide’s low solubility, reflect a combined effect of slow drug release from the device and partitioning into the lipophilic retina.

We chose to evaluate the pharmacokinetics of the fluocinolone acetonide device in normal rabbit eyes so that any drug-related toxic effects could be correlated with intravitreal drug levels and would not be confounded by intraocular inflammation. It is possible that the pharmacokinetics of the device would differ in normal and inflamed eyes. Experiments to determine device pharmacokinetics in inflamed eyes were beyond the scope of the current studies. However, such studies would provide clinically relevant data and would be worth pursuing in future investigations.

In this study, we did not specifically test the fluocinolone acetonide device efficacy. Furthermore, the levels necessary to produce a therapeutic effect in the treatment of chronic diseases such as uveitis are unknown. However, based on fluocinolone acetonide’s lipophilicity, the potency of fluocinolone acetonide, which is similar to that of dexamethasone, the efficacy of the dexamethasone device in animals3,4 and in a human, and preliminary evidence that the fluocinolone acetonide device is effective in patients,11 we anticipate that the fluocinolone acetonide drug delivery device will be an effective method to treat uveitis. Experiments are currently underway in our laboratory to further test the fluocinolone acetonide device efficacy.

The fluocinolone acetonide device was not toxic by any of the measurement parameters. Similarly, we did not observe toxicity from the dexamethasone sustained drug delivery device.8 However, in that study, to create a dexamethasone device that would release drug over a period comparable to that of the fluocinolone acetonide device would have required a device that would have been so large as to be impractical clinically. Thus, a direct comparison of chronic fluocinolone acetonide device toxicity to that of the dexamethasone device is not possible.

In patients, extended corticosteroid use may be associated with cataract formation. The lack of cataract observed in our study is encouraging. However, species differences preclude extrapolation of our data from rabbits to humans. Furthermore, device toxicity was tested in normal rabbit eyes. It is recognized that potential toxic effects may differ in normal and diseased eyes (e.g., those with uveitis). However, in these experiments, we chose to study normal eyes so that it would be easier to differentiate drug-induced effects from those caused by the disease itself. These initial studies provide a basis for future experiments to evaluate the fluocinolone acetonide device in eyes with preexisting ocular pathology.

The b-wave amplitude ratios measured by electroretinography were slightly greater than 1 at early time points. The reason for this observation is unclear. However, the ERG results both at early and later time points are consistent with the lack of retinal toxicity observed clinically or histopathologically. They are also consistent with results observed in our previous studies of a dexamethasone sustained drug delivery device.3,4 In those studies, not only was the dexamethasone device nontoxic to the normal rabbit retina, but the device preserved retinal function in a model of severe uveitis as determined electroretinographically and histopathologically.4 The lack of observed toxicity with the fluocinolone acetonide device is reassuring because it is likely that fluocinolone acetonide will partition into the lipophilic retinal tissues as described above.

In humans, corticosteroids may cause increased intraocular pressure. Aqueous fluocinolone acetonide levels were undetectable during the course of the study. One might anticipate that the low fluocinolone acetonide levels measured in the current experiments would minimize a tendency for corticosteroid-induced increased intraocular pressure. However, adult rabbits do not develop corticosteroid-induced increased intraocular pressure in a reproducible manner.12 Furthermore, we tested the fluocinolone acetonide device in normal eyes, and the tendency to produce increased intraocular pressure (or other secondary effects) might differ in an eye with ocular inflammation or other ocular diseases. Accordingly, it is not possible to draw definitive conclusions regarding the ocular hypertensive potential of the fluocinolone acetonide device. In any case, in initial clinical trials, it will be important to exclude patients with uncontrolled corticosteroid-induced increased intraocular pressure.

References


