Pharmacokinetic and Toxicity Study of an Intraocular Cyclosporine DDS in the Anterior Segment of Rabbit Eyes

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PURPOSE. To establish the safety and pharmacokinetic efficacy of an Oculex Drug Delivery System (DDS; Oculex Pharmaceuticals, Inc., Sunnyvale, CA) containing cyclosporin A (CsA) in the anterior segment of the rabbit eye.

METHODS. The Oculex DDS is an intraocular, sustained-release, drug delivery system comprising a biodegradable lactic acid-glycolic acid copolymer. A controlled prospective study was performed that involved implanting a DDS containing 0.5 mg of CsA into the anterior chamber (AC) of the right eyes of 16 New Zealand White rabbits. A placebo DDS was implanted into the left eyes of these same rabbits as the control. Slit lamp examinations and AC taps were performed serially, and the rabbits were killed and the globes removed at 2, 4, 8, and 12 weeks for histology and determination of CsA drug levels. Analysis of CsA levels was performed with high-performance liquid chromatography–mass spectrometry.

RESULTS. High concentrations of CsA were detectable in all layers of the cornea (epithelium, corneal stroma and endothelium) throughout the 3-month period. Low CsA levels were detected in the aqueous, whereas no CsA was detectable in the blood. There were no adverse reactions observed.

CONCLUSIONS. The Oculex DDS CsA device is effective in delivering long-term levels of CsA to corneal tissues, without adverse effects. Further studies in an animal model of corneal transplant rejection should be performed to determine the potential of this device in the prophylaxis and treatment of corneal transplant rejection in humans. (Invest Ophthalmol Vis Sci. 2003;44:4895–4899) DOI:10.1167/iovs.02-1112

Cyclosporin A (CsA) is a potent immunomodulatory drug that has been used to prevent immune rejection in high-risk corneal transplantation.1–3 It has also been favored for ophthalmic use as an anti-inflammatory steroid-sparing agent in inflammatory eye diseases, such as severe sight-threatening posterior uveitis,4 and most recently, in the treatment of ocular surface inflammation, dry eye, and allergic eye disease.5–10 Although systemic CsA has clear therapeutic efficacy, its use is limited by the potential for systemic side-effects—most notably, nephrotoxicity and hepatotoxicity—and its penetration into ocular tissues at a therapeutic level is believed to occur only in significantly inflamed eyes.11,12

Topical CsA preparations traditionally involve the use of an oil-based agent, because the drug is hydrophobic. Some studies suggest that topical CsA 2% achieves immunosuppressive levels of activity in the cornea and less significantly in some ocular compartments (anterior segment) but not in the posterior segment of the eye.11,15 It may be prepared as a 2% ointment, or more recently, as a suspension of 0.5%, and is generally used for the treatment of ocular surface inflammatory disease, dry eye, ocular surface allergy and corneal graft rejection,14 but the efficacy of topical CsA remains controversial.11,15 Topical CsA may also cause toxic keratitis, which limits its use in ocular surface disease and corneal transplants.

The Oculex Drug Delivery System (DDS; Oculex Pharmaceuticals, Inc., Sunnyvale, CA) is a biodegradable system that provides sustained drug release after insertion into the eye at the conclusion of surgery. The DDS consists of an active drug plus a biodegradable lactic acid/glycolic acid copolymer (poly[Delta-lactide-glycolide]), which has been used as a synthetic absorbable suture material in general and oculary surgery as Vicryl (Ethicon, Piscataway, NJ). This polymer has been shown to degrade safely by hydrolysis into the natural byproducts lactic acid and glycolic acid, which are metabolized. Surodex was the first DDS formulation from Oculex Pharmaceuticals and contained 60 μg dexamethasone designed to reduce postoperative inflammation after cataract surgery. We have performed two randomized, controlled human clinical trials comparing Surodex, with conventional 0.1% dexamethasone eye drops in patients who have undergone cataract surgery.16,17 Results showed that placement of one or two Surodex implants in the anterior chamber (AC) is significantly more effective than eye drops in reducing aqueous flare levels, and Surodex was found to be safe, with no complications related to Surodex insertion found in analyses including endothelial cell counts a year after surgery.

A similar DDS bearing CsA, which is able to release CsA into the AC and corneal tissues over a prolonged period, may be of use in corneal transplantation as a safer means of localized anti-inflammatory and immunomodulatory treatment, to reduce the incidence and treat corneal transplant rejection, as well as in the management of uveitis and ocular surface inflammation, as opposed to conventional systemic CsA treatment. The objectives of this study were to evaluate the safety, duration, and pharmacokinetic efficacy of a DDS containing CsA, when implanted in the AC of the rabbit eye.

METHODS. This was a prospective, placebo-controlled study comparing the effect of inserting a DDS containing 0.5 mg of CsA into the anterior segment of rabbit eyes, with that of a placebo DDS. The study was conducted in compliance with the ARVO Statement for the Use of Animals in Investigative Ophthalmology & Visual Science, November 2003, Vol. 44, No. 11

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Surgical Procedure
The DDS CsA pellet, measuring 1 × 2 mm, was implanted into 16 right eyes of 16 New Zealand White rabbits (Fig. 1) with a placebo DDS implanted into the 16 left eyes of these same rabbits as the control. Before surgery, the rabbit’s eyes were treated with prophylactic topical ciprofloxacin HCl 0.3%, (Ciloxan; Alcon-Couvreur, Puurs, Belgium) four times a day, 1 day before surgery. The rabbits were anesthetized with intramuscular ketamine followed by intravenous injection of ketamine (50 mg/kg). The right eyes were then cleaned with povidone iodine and drapped. Topical amethocaine was applied, and a small (1.5-mm) corneal tunnel was created at the superotemporal limbus using a phacoemulsification keratome. Sodium chondroitin sulfate-sodium hyaluronate (Viscoat; Alcon Surgical, Alcon-Couvreur) was injected to reform the AC. The CsA DDS was inserted through the corneal tunnel into the inferior temporal chamber angle, and the limbal wound was closed with one 10-0 nylon stitch, and the AC reformed with saline solution (Balanced Salt Solution; BSS; Alcon-Couvreur). Residual viscoelastic was not removed. A similar procedure was then performed in the fellow (left) eye, and a DDS placebo was inserted to act as the control. Subconjunctival gentamicin (10 mg/0.25 mL) was given at the end of the procedure in each eye. After surgery, topical ciprofloxacin was applied four times a day for 1 week as a prophylaxis.

Clinical Assessment
Clinical observations were made before and after the procedures, and the behavior of the device in the anterior segment was monitored with respect to migration, effect on the cornea, AC inflammation, and intraocular pressure. Slit lamp examinations were performed before surgery and on days 1, 4, 7, and 14 and at 2-week intervals thereafter until 12 weeks after surgery. A final examination was performed before the rabbits were killed. Records of the slit lamp examination of the anterior segments included corneal clarity, the degree of AC activity, iris, DDS device, and lens clarity. Intraocular pressure (IOP) was measured using a handheld tonometer (Tonopen; Mentor, Norwell, MA) before implantation and after each slit lamp examination was performed. Fundus examination was performed at 1 week and at the time of death.

Pharmacokinetic and Histologic Analysis
Aqueous taps were performed 1 week after surgery and at the time of death, and each sample was assayed for CsA levels by high-performance liquid chromatography–mass spectrometry (LC-MS).

Treatment subjects were divided into four groups consisting of four rabbits each. The rabbits in each group were killed at different time intervals: 2 weeks, 1 month, 2 months, and 3 months after the insertion of the DDS CsA into the anterior segment. The rabbit globes (eight eyes per group, four containing active DDS CsA and four containing placebo) were removed to enable full toxicologic and histologic analyses. Rabbits were anesthetized, 2 mL of blood was taken, and 0.1 mL of aqueous together with a piece of superior conjunctiva was removed from each eye. The rabbits were then killed and the eyes enucleated. One half of the entire cornea including the limbus (the section which excluded the wound site) was removed, leaving the other half intact and attached to the globe. The limbus was separated from the detached corneal half, and the corneal epithelium and endothelium stripped from the corneal stromal remnant. The limbal tissue, corneal epithelium, corneal stroma, and corneal endothelium were immediately weighed by using a microbalance before freezing in liquid nitrogen and stored at −80°C for subsequent evaluation of CsA levels by LC-MS. In addition, a segment of iris and a vitreous sample was similarly removed from the globe, weighed, and stored for LC-MS analysis.

The globe remnant was fixed in 10% buffered formalin and sent for histologic sectioning, with identification of the 10 o’clock location of the corneal surgical site and implantation site of DDS units in the AC by the presence of the corneal suture placement. At tissue trimming, eyes were fixed, processed, blocked and sectioned and tissue sections stained with hematoxylin and eosin. At sectioning, all eyes were oriented to obtain tissue sections through the optic nerve, central plane of the iris, and the corneal surgical sites. Tissue sections from each eye were examined by light microscopy for histopathologic changes.
High-Performance Liquid Chromatography–Mass Spectrometry

The HPLC grade methanol was purchased from Fisher Scientific (Pittsburgh, PA); Ultrapure water was obtained from a water purification system (Milli-Q; Millipore, Milford, MA). CsA was a gift from Novartis Pharma AG (Basel, Switzerland).

Each corneal tissue specimen was extracted by vortexing with 200 μL of methanol. The insoluble protein was removed by centrifugation. The soluble fraction was used for CsA analysis. For Humor and vitreous humors, 40–50 mL samples were extracted by 80 μL ethyl acetate. The upper organic layer was transferred to a glass vial and evaporated to dryness in a freeze dryer. The residue was dissolved in 40 μL methanol and 30 μL was injected into the LC-MS system.

The LC-MS system including a Waters 2690 solvent delivery system, an auto-sampler, photodiode array detector (Waters Associates, Milford, MA), and Micromass Mass-Stacker-Platform LCZ (Micromass, Manchester, UK), was used for chromatographic separation and detection of CsA. The separation column, RP18 (3.5 μm, 4.6 × 75 mm supplied by Waters Associates) was eluted by 90% methanol and 10% water at 0.5 mL/min.

The optimized settings in the MS detector were as follows. The nitrogen gas flow was maintained at 500 L/h. The capillary and cone voltage was set to 4.0 and 10.0, respectively. The source and desolvation temperatures were set to 150°C and 357°C, respectively. The mass spectrum was recorded under a full-scan operation for positive ions, with a scan range from m/z 500 to 1400. The quantification was performed with the selected-ion recording (SIR) mode by monitoring the protonated molecular ion (m/z = 1203), sodium ion adduct (m/z = 1241), and potassium ion adduct (m/z = 1241) simultaneously. The quantification is based on the total peak areas of CsA–H+, CsA–Na+, and CsA–K+ in an SIR chromatogram.

The determination of CsA was based on the external standard method. Six-point calibration curves (triplicate injections) were created for the range from 0.02 to 10 ng by plotting the summation of the peak areas of CsA–H+, CsA–Na+, and CsA–K+ against the amount of CsA injected into the column. Others and we have previously shown that the technique of LC-MS used in this study to determine precise levels of CsA in the ocular tissues is a highly sensitive method for the analysis of CsA in pathologic specimens.16,19

Evaluation of CsA in Corneas of Humans on Systemic CsA

To compare these levels of CsA in the rabbit corneas achieved with DDS CsA, with levels that may be achieved with systemic CsA in human corneas, we measured the levels of CsA in the corneal buttons of eight patients who had been in therapy with 4 mg/kg body weight CsA (Neoral, Novartis) for 3 days before having penetrating keratoplasty performed. The cornea buttons were removed during the time of surgery, in accordance with the provisions of the Declaration of Helsinki, and sent to the laboratorv for LC-MS analysis for CsA. The technique of stripping the cornea buttons into epithelium, stroma, and endothelium and the analysis for CsA were exactly the same as for the rabbit corneas.

RESULTS

Clinical Evaluation

Clinical slit lamp biomicroscopy was performed on postoperative days 1, 4, 7, 14, 28, 42, 56, 70, and 84 until the time of death to evaluate the status of the cornea, AC cells, flare, iris, and the retina. Mild cornea edema around the wound was noted in almost all eyes after surgery. This resolved within 4 days to 2 weeks. Mild iris anterior synechiae at the wound site, related to difficulty with localized maintenance of the AC at the time of surgery was seen in half the cases. The rest of the corneas remained clear. In the last few cases, in which surgical time was shortest, no cornea edema was noted around the wound. In one case, severe diffuse corneal edema was seen at 2 weeks after surgery, and it was associated with a spike of intraocular pressure (IOP) ranging from 20 to 40 mm Hg as measured with a handheld tonometer (Tonopen; Mentor). The mean preoperative IOP of all 16 rabbits (n = 32) was 11.3 mm Hg with a range from 7 to 21 mm Hg. The corneal edema resolved at approximately 2 months and was later found to be due to a total Descemet’s membrane detachment, probably induced at the time of surgery. No histologic evidence of inflammation and necrosis was seen in that case. No other eyes experienced an increase in IOP.

The anterior segments were generally quiet in both the right and left eyes of all rabbits. Fibrin was noted in all cases within the first 1 to 2 weeks of insertion of the pellets, with no difference noted between CsA- and placebo-treated eyes. AC cells were not seen in both eyes in all cases, even during the early postoperative phase when fibrin was present.

A mild fibrin reaction around the pellet occurred almost immediately at the point of surgical insertion and persisted for approximately 1 to 2 weeks after surgery. This reaction was associated with mild peripheral iris vascular engorgement at the site of pellet placement and was noted between 2 weeks to 2 months in both the CsA- and placebo-treated eyes. It resolved with dissolution of the pellet. This vascular engorgement was not associated with cornea edema, increased IOP, or any increase in the presence of fibrin in the AC.

The rate of dissolution appeared to differ significantly between CsA and control pellets, with the CsA DDS generally persisting longer than the placebo. The CsA DDS generally remained intact during the first few weeks, showed surface changes in dissolution only after 6 weeks of insertion into the AC, and showed a reduction in size by 10% to 20% at the 3-month review. The placebo, by contrast, dissolved much more rapidly, and evidence of dissolution was seen as early as the first postoperative day. Approximately 40% to 60% of the placebo was dissolved by the end of 1 to 2 months. By the end of 3 months 4 of 16 placebos had completely dissolved, and the other eyes showed remnants, which were 10% to 30% of the original pellet size. One possible explanation for the apparently slower dissolution rate of the CsA DDS is that CsA is lipid soluble, which may block the polymer matrix and slow down the dissolution or degradation of the polymer matrix.

No abnormal findings were seen in the crystalline lens or fundi before or after the introduction of DDS in the AC in any of the tested animals. Wounds in all cases were intact, with eventual focal scarring. No wound infection or endophthalmitis occurred in any case.

Histologic Evaluation

Histologic sections revealed the presence of focal lesions confined to the cornea and iris at the surgical wound sites, consistent with the surgery. These included focal fibromas around the sutures and incomplete healing of focal disruptions of Descemet’s membrane and anterior peripheral iris synchiae: there was no difference between CsA and control eyes. No abnormal histologic findings were noted in relation to the CsA eyes at any time point, and cornea, sclera, iris, ciliary body, lens choroid, retina, and optic nerves were normal.

Pharmacokinetic Analysis

There was no CsA detected in all tissues obtained from the control eyes. No CsA was detected in all samples of rabbit plasma, including subjects with a CsA DDS implanted. In eyes in which DDS CsA devices were implanted, the levels of CsA detected in the aqueous humor ranged from 11.57 to 43.71 ng/mL with the highest concentrations achieved after 1 week.
DISCUSSION

Although rarely used in routine keratoplasty, systemically administered CsA has increased the survival of high-risk corneal transplants significantly.20,21 However, systemic treatment with immunosuppressive agents may produce severe systemic side effects and thus limit their use in corneal transplantation. Although the efficacy of topical CsA treatment is controversial, topical application of CsA has been reported to prolong successfully the survival of corneal allografts in rabbits.22,23 In the study by Foets et al.,22 all grafts in rabbits treated with low-dose topical CsA remained clear, whereas the untreated control grafts were rejected. Similar application to the contralateral eyes led to all grafts failing, despite similar systemic CsA levels suggesting that the mechanism of action of topical CsA involved primarily local immunosuppression rather than a general systemic effect. Furthermore, Zhao and Jin24 demonstrated that treatment of corneal graft rejection with 0.5% topical CsA yields good results in human eyes. Although the mechanisms leading to corneal allograft rejection are still not fully understood, evidence suggests that ocular immunologic components as part of the immune system play a dominant role in the course of graft rejection.22,24,25 Immune cells of ocular tissue recognize foreign antigen, leading to host immune system sensitivity, which results in an immune reaction and subsequently, tissue damage. We therefore embarked on this study to determine the safety and pharmacokinetic efficacy of a CsA DDS in the rabbit eye, with a view toward its potential anti-inflammatory and/or local immunomodulatory use in the prevention or treatment of corneal allograft rejection.

Our histology results and clinical evaluations suggest that implantation of the DDS CsA in the anterior segment of the rabbit eyes is safe. Apart from the case of surgically induced total Descemet’s detachment that was encountered, no clinically detectable adverse events were noted in relation to the treatment. Although clinically detectable engorgement of blood vessels in the iris in both eyes containing active CSA in the right eye and placebo in the left were noted, this appeared to be related to the visible presence of the pellet and was not accompanied by any detectable histologic evidence of inflammation or necrosis. The presence of fibrin around the DDS CsA was to be expected, because increased inflammation is frequently seen in rabbit eyes compared with human eyes but despite that, all fibrin accumulations resolved after 2 weeks of implantation of the DDS CsA or placebo.

Our pharmacokinetic results show that this sustained-release device is capable of producing high levels of CsA in the corneal endothelium, epithelium as well as stroma of rabbit eyes beyond 12 weeks. The concentration of CsA in iris tissue is relatively low. The reason could be that there are few binding sites on the iris tissue or that the pathways by which CsA is eliminated are very efficient. By 2 weeks, the initial equilibrium has already been established. It is interesting to see that the corneal epithelium contains the highest levels of the CsA among the three layers of cornea (epithelium, stroma, and endothelium). A possible explanation for this is that the corneal epithelium layer contains more cells per tissue weight unit (higher cell density) and hence more binding sites per tissue weight unit, and it is also the end point for diffusion along the

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**Table 1. Average Concentrations of CsA in the Various Tissues of the Rabbit Eye at Different Intervals**

<table>
<thead>
<tr>
<th>Time</th>
<th>Epithelium</th>
<th>Stroma</th>
<th>Endothelium</th>
<th>Limbus</th>
<th>Iris</th>
<th>Conjunctiva</th>
<th>Vitreous (ng/mL)</th>
<th>Aqueous (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 weeks</td>
<td>32.7 ± 14.6</td>
<td>5.41 ± 1.08</td>
<td>20.4 ± 13.7</td>
<td>0.95 ± 0.32</td>
<td>1.17 ± 0.38</td>
<td>0.16 ± 0.16</td>
<td>—</td>
<td>43.71 ± 14.6</td>
</tr>
<tr>
<td>4 weeks</td>
<td>30.1 ± 8.6</td>
<td>3.13 ± 0.98</td>
<td>6.17 ± 3.02</td>
<td>0.47 ± 0.32</td>
<td>0.64 ± 0.48</td>
<td>0.03 ± 0.06</td>
<td>—</td>
<td>11.57 ± 1.82</td>
</tr>
<tr>
<td>8 weeks</td>
<td>19.9 ± 6.5</td>
<td>2.29 ± 0.66</td>
<td>5.49 ± 4.96</td>
<td>0.53 ± 0.24</td>
<td>0.51 ± 0.39</td>
<td>0.00 ± 0.00</td>
<td>—</td>
<td>14.36 ± 5.37</td>
</tr>
<tr>
<td>12 weeks</td>
<td>25.0 ± 9.9</td>
<td>2.47 ± 0.72</td>
<td>3.53 ± 1.05</td>
<td>0.46 ± 0.21</td>
<td>0.63 ± 0.41</td>
<td>0.08 ± 0.16</td>
<td>9.64 ± 2.79</td>
<td>11.69 ± 2.05</td>
</tr>
</tbody>
</table>

Tissue concentrations are expressed as mean nanograms/milligram tissue weight ± SD. n = 4.

**Table 2. Concentrations of CsA in the Corneal Buttons of Patients on 4 mg/kg Body Weight Cyclosporine, after Corneal Graft Surgery**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelium</td>
<td>0.74</td>
<td>3.53</td>
<td>4.21</td>
<td>3.75</td>
<td>2.15</td>
<td>1.27</td>
<td>0.76</td>
<td>1.35</td>
<td>2.20 ± 1.41</td>
</tr>
<tr>
<td>Stroma</td>
<td>7.98</td>
<td>0.00</td>
<td>0.15</td>
<td>0.11</td>
<td>0.00</td>
<td>0.00</td>
<td>0.40</td>
<td>0.05</td>
<td>1.09 ± 2.79</td>
</tr>
<tr>
<td>Endothelium</td>
<td>0.68</td>
<td>0.00</td>
<td>0.07</td>
<td>1.50</td>
<td>0.23</td>
<td>0.10</td>
<td>0.00</td>
<td>0.09</td>
<td>0.35 ± 0.52</td>
</tr>
</tbody>
</table>

Concentrations are expressed as nanograms/milligram tissue weight.
forward pathway (CsA-aqueous humor-corneal endothelium-corneal stroma-corneal epithelium).

The results of the levels of CsA in the human corneal buttons of the eight patients who had cornea transplants and who were on systemic CsA show some variations in the concentrations of CsA and may be due to differences in the degree of corneal neovascularization or anterior segment inflammation present in these eyes at the time of corneal transplantation. The very high levels of CsA in the stroma in case 1 were in a patient who had two previous failed grafts with an opaque and fully vascularized cornea and an ulcer with a small descemetocele of 3 days’ duration that required urgent corneal graft surgery. This could explain the very high concentration of CsA in the stroma, as CsA is likely to have gained direct entry into the corneal stroma through the ulcer from the tear film and from the blood vessels in the cornea extending from the limbus. Although fairly large amounts of CsA were found in the stroma of all specimens, because the stroma is much thicker than the epithelium and endothelium, the amount of CsA per milligram of tissue (concentration) showed the lowest levels among the three layers of the cornea. Comparatively, the concentrations of CsA in rabbit corneas from DDS CsA were much higher that those achieved in the human corneas ($P < 0.0001$).

The implications of a long-acting anterior segment depot of topical CsA has potential treatment modalities for not only corneal transplantation rejection prophylaxis, and treatment, but also for other forms of anterior segment inflammatory disorders that may require a prolonged anti-inflammatory regimen. Conditions such as chronic immune-mediated stromal keratitis and chronic anterior uveitis may thus benefit from CsA DDS implantation as a potential steroid-sparing agent. Studies are currently underway to determine the exact duration of CsA DDS and to evaluate the efficacy of CsA DDS in the treatment of allograft rejection in an animal corneal transplantation model.

References