Sustained Intraocular Rapamycin Delivery Effectively Prevents High-Risk Corneal Allograft Rejection and Neovascularization in Rabbits

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PURPOSE. To evaluate the immunosuppressive and antiangiogenic activities of an intraocular rapamycin (RAPA) drug delivery system (DDS) in a rabbit model of high-risk penetrating keratoplasty.

METHODS. Forty New Zealand White rabbits with corneal neovascularization underwent allograft cornea transplantation and were randomly divided into four groups: a control group, a glycolide-co-lactide-co-caprolactone copolymer (PGLC)–implanted group, a RAPA eye drop group, and a RAPA-PGLC DDS–implanted group. Graft survival, corneal neovascularization, and RAPA concentration in the aqueous humor were monitored for 90 days. Corneal grafts were also examined by in situ hybridization and immunohistochemistry for proinflammatory gene expression.

RESULTS. In the control and PGLC groups, graft rejection occurred within 3 weeks of keratoplasty. In the RAPA eye drop and RAPA-PGLC groups, corneal rejection was significantly delayed, and neovascularization was markedly inhibited. Median graft survival times were 36 and >90 days in the eye drop and RAPA-PGLC groups, respectively. Mean RAPA concentrations in the aqueous humor were 10.7 ng/mL, 12.0 ng/mL, 9.2 ng/mL, and 7.0 ng/mL in the RAPA-PGLC group 2, 4, 8, and 12 weeks after surgery, respectively. By contrast, RAPA was undetectable in the aqueous humor in the eye drop group. High levels of IL-2R, MCP-1, TNF-α, and VEGF were detected in the corneal grafts of the control and PGLC groups but not in those of the RAPA-treated groups.

CONCLUSIONS. RAPA-PGLC DDS and RAPA eye drops can significantly prolong the survival of allografts at high risk and inhibit corneal neovascularization. However, RAPA-PGLC DDS is far more effective than RAPA eye drops in preventing corneal graft rejection. (*Invest Ophthalmol Vis Sci.* 2006;47:3339–3344) DOI:10.1167/iovs.05-1425

Allograft rejection is the leading cause of corneal graft failure, and corneal neovascularization is the most common risk factor associated with corneal graft rejection.[1] To inhibit allograft rejection and to avoid the potential adverse effects of corticosteroids, a number of new immunosuppressive agents, such as cyclosporin (Cs)A, tacrolimus (FK506) and rapamycin (RAPA; sirolimus), have been developed. These immunosuppressive compounds act in a similar fashion inside the cell. They bind to the same family of intracytoplasmic regulatory proteins known as immunophilins. These proteins catalyze the folding of proline-containing polypeptides and, therefore, have peptidyl-prolyl isomerase activities. The exact mechanism of immunosuppression after the binding of immunophilins is not yet clear. It is known that CsA and FK506 bind to different immunophilins but that they have virtually indistinguishable immunosuppressive effects. On the other hand, though FK506 and RAPA bind to the same protein, they have different effects on the immune system.[2,3]

RAPA is a carbocyclic lactone–lactam macrolide antibiotic with strong immunosuppressive properties. It is produced by *Streptomyces hygroscopicus* and was first isolated in 1975 from a soil sample taken from Easter Island.[4] In vitro studies have shown that RAPA is up to 100 times more potent than CsA. More important, it can inhibit the neovascularization associated with tumor. RAPA was shown in animal and human clinical trials to inhibit solid organ allograft rejection.[5-8] However, to date, RAPA can only be administered systemically. Its intraocular application has been hindered by three key problems. First, RAPA cannot be easily converted to a palatable substance. Second, the pharmacologic kinetics profile of RAPA is low (approximately 15%), and the therapeutic window of RAPA is narrow.[9] Third, RAPA is a hydrolyzed, oxidized compound that is prone to decomposition on exposure to light, which makes stabilization for medical use difficult.[10]

Implantable drug delivery systems (DDSs), which can be placed in the eye during eye surgery, can penetrate the blood-aqueous barrier and confer a stable drug concentration in aqueous humor. In this study, we developed a RAPA–glycolide-co-lactide-co-caprolactone copolymer (PGLC) DDS that steadily releases the drug into aqueous humor. The device, which is made of biodegradable PGLC, can be implanted at the anterior chamber angle through a simple procedure. We report here that this new RAPA delivery system is highly effective for preventing corneal allograft rejection and neovascularization in rabbits at high risk for these occurrences.

MATERIALS AND METHODS

Animals

Sixty inbred adult New Zealand White rabbits, weighing 2.0 to 2.5 kg each, were used in this study. Neovascularization was induced in the cornea of each right eye of 40 rabbits by placing a 5-0 silk suture in each of the cornea quadrants. Animals were examined by slit lamp biomicroscopy three times a week, and the sutures were removed under topical anesthesia 2 weeks later when superficial vessels had grown at least 4 mm into the cornea in three or more quadrants. The

remaining 20 rabbits were used as cornea donors. This study was approved by the Institutional Animal Care Committee, and all procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

RAPA Eye Drops

RAPA powder (99% purity; North China Pharmaceutical Group, Shijiazhuang, China) was mixed with soybean oil (Huanye Pharmaceutical Co., Guangzhou, China) by agitation for 12 hours to produce the 1% RAPA eye drop suspension. The suspension was stored in a sterile amber-colored bottle at 4°C and was mixed by shaking before each use. RAPA eye drops were used within 1 week of production.

RAPA-PGLC DDS

We prepared RAPA-PGLC DDS using PGLC as the carrier (provided by the Institute of Chemistry, Chinese Academy of Sciences, Beijing, China). Briefly, 50 mg PGLC was mixed with 50 mg RAPA and was lyophilized with acetone ice and a vacuum pump. The resultant mixture was compressed into small films (length, 3.0 mm; width, 1.0 mm; thickness, 0.5 mm) that weighed 1.0 mg and contained 0.5 mg RAPA each. Control films were prepared in the same manner but with PGLC only. Film devices were sterilized with ethylene oxide for 48 hours and then stored in a desiccator at 4°C. All devices were used within 2 weeks of their preparation.

Surgery

Surgical techniques for penetrating keratoplasty (PK) were previously described.[11] General anesthesia was achieved by intramuscular injection of ketamine (25 mg/kg) and chlorpromazine (25 mg/kg). Donor rabbits were sterilized, and a 7.5-mm punch was used to produce a corneal button graft. Recipient corneas were prepared according to the same procedure but with a 7.0-mm biopsy punch. Donor grafts were joined to the recipient corneas with a 10–0 monofilament nylon suture. PGLC carrier and RAPA-PGLC DDS were implanted into the anterior chamber at the end of the surgery, and the anterior chamber was reconstructed with the balanced sodium solution. Heparin (1000 U/mL) was applied topically to prevent the formation of aqueous clots in the anterior chamber during the surgery. Ofloxacin eye ointment (0.3%) was applied at the end of the procedure and once a day for 3 consecutive days. Sutures were removed 14 days after surgery.

Clinical Evaluation

Two investigators examined corneal allografts by slit lamp microscopy each day for the first 14 days and twice a week thereafter. A rejection index (RI), on a scale of 0 to 12, was calculated based on the combined scores of three allograft indicators—clarity, edema, and neovascularization—each of which was scored on a scale of 0 to 4. RIs of animals in each group were averaged to generate a mean RI that represented the overall allograft status of each group. The scoring system used was as follows[12,13]: for clarity: 0, clear cornea; 1, slight haze; 2, increased haze but anterior chamber structures still clear; 3, advanced haze with difficult view of the anterior chamber; 4, opaque cornea without view of the anterior chamber; for edema: 0, no stromal or epithelial edema; 1, slight stromal thickness; 2, diffuse stromal edema; 3, diffuse stromal edema with microcystic edema of epithelium; 4, bullous keratopathy; for neovascularization: 0, no vascularization at graft-host junction (GHJ); 1, vascularization at GHJ in one quadrant only; 2, vascularization at GHJ in two quadrants only; 3, vascularization at GHJ in three quadrants only; 4, vascularization at GHJ in all four quadrants.

Assay of RAPA in Aqueous Humor

At 2, 4, 8, and 12 weeks after PK, rabbits in the RAPA eye drop and the DDS groups were anesthetized, and 100 μL aqueous humor was collected from each. High-performance liquid chromatography (HPLC) was used to determine RAPA concentration in the aqueous humor.

Histology, In Situ Hybridization, and Immunohistochemistry

Grafts were examined using standard histology, in situ hybridization, and immunohistochemical methods for 16 rabbits: four untreated, four from the PGLC carrier group, four from the RAPA eye drop group, and four from the RAPA-PGLC DDS group. Allografts from the control and PGLC carrier groups were collected at the time of maximal rejection (day 20); edema and heavy neovascular invasion were observed in them. Allografts from the RAPA eye drop and DDS groups were also taken at day 20 after PK. Serial olefin sections (5 μm) of each eye were prepared for immunohistology and in situ hybridization studies. Adjacent olefin tissue sections were stained with hematoxylin and eosin. Ultrastructural studies were carried out on the procured specimens of eyeball, liver, and kidney at day 90 after PK.

Tissue specimens were subjected to in situ hybridization for the detection of interleukin-2 receptor (IL-2R) and monocyte chemotactic protein-1 (MCP-1) transcripts with nonradioactive probes.[14] After prehybridization with 50% deionized formamide 2×SSC for 1 hour at 45°C, the specimens were hybridized with 0.5 mg/mL antisense or sense probe in 50% deionized formamide, 2.5 mM EDTA (pH 8.0), 300 mM NaCl, 1× Denhardt solution, 10% dextran sulfate, and 1 mg/mL brewer's yeast tRNA at 45°C for 16 hours. After hybridization, the slides were washed in 50% formamide 2× SSC for 1 hour at 45°C and were digested with 10 mg/mL RNase A at 37°C for 30 minutes. They were further washed sequentially with 2× SSC/50% formamide at 45°C for 1 hour, 1× SSC/50% formamide at 45°C for 1 hour, and 1× SSC/50% formamide at room temperature for 30 minutes. No specific reactivity was observed in the control group using the sense probe. Photographs were taken under bright-field and dark-field illumination. All reagents were purchased (Wuhan Boster Biological Technology Co., Wuhan, China).

Tissue specimens were also subjected to fluorescence immunohistochemistry for the detection of VEGF and TNF-α. Tissue sections were pretreated with an enhancer (Histosafe; Linaris, Bettingen, Germany) to improve antibody affinity. Primary monoclonal antibody was obtained (Santa Cruz Biotechnology, Santa Cruz, CA), as was the secondary antibody, fluorescence-conjugated goat anti–mouse immunoglobulin G (Wuhan Boster Biological Technology Co.). Slides were washed three times in phosphate-buffered saline. Slides were then examined and images were collected through a fluorescence microscope (E800; Nikon, Tokyo, Japan) with an attached camera.

Statistical Analysis

The Kaplan-Meier method was used to compare the survival curves of the allografts. Log-rank test was used to compare the survival curves between groups. Clinical rejection index (RI) and neovascularization index scores were compared with the Mann-Whitney *U* test.

Results

Superiority of RAPA-PGLC DDS over RAPA Eye Drops for Preventing Corneal Allograft Rejection

Forty rabbits were randomly divided into four groups consisting of 10 animals per group. Group A, the control group, received no treatment. Group B, the PGLC carrier–implanted group, received 1 mg PGLC carrier implantation in the anterior chamber. Group C, the RAPA eye drop group, received 1% RAPA eye drops four times a day throughout the study. Group D, the RAPA-PGLC DDS–implanted group, received 1 mg RAPA-PGLC DDS (containing 0.5 mg RAPA) implantation in the anterior chamber. All rabbits underwent corneal transplantation, as described in Materials and Methods.

After surgery, mild corneal edema was seen in all animals and disappeared 3 days after transplantation. In all treatment groups, enlarged blood vessels were seen around the graft. In the control and PGLC carrier groups, opacification peaked on
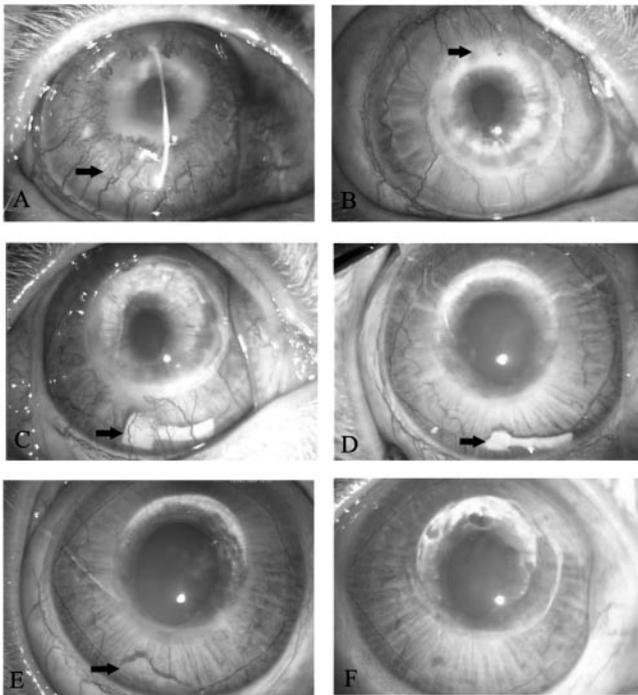
FIGURE 1. RAPA-PGLC DDS prevents cornea rejection. (**A**) Rejected corneal allograft in the control group on postoperative day 14. Note the corneal edema and neovascularization. (**B**) Clear corneal allograft with neovasculature invading graft–host junction in one quadrant (*arrow*) in the RAPA eye drop group on postoperative day 14. (**C–F**) Clear corneal allografts in the RAPA-PGLC DDS group on postoperative days 14, 60, 90, and 120, respectively. Note that the DDS dissolved gradually.

the 16th day with a large amount of neovasculature invading the grafts in most quadrants (Fig. 1A). In the RAPA eye drop–treated group, the graft remained clear until 30 days after PK, with less neovasculature moving into the grafts (Fig. 1B). In the RAPA-PGLC DDS group, 8 of 10 grafts remained clear for more than 90 days. RAPA-PGLC DDS underwent gradual biodegradation (Figs. 1C–F). We extended the follow-up time to more than 120 days in two rabbits, when the DDS was no longer visible by slit lamp microscopy (Fig. 1F).

Figure 2 depicts a Kaplan-Meier survival curve comparing allograft survival in all rabbits. Allografts with RIs of 6 or greater were considered rejected. All grafts in the control and PGLC-implanted groups were rejected within 3 weeks. Survival time medians of group A and group B were 16.0 ± 0.5 and 15.0 ± 0.8 days, respectively. Animals given the PAPA eye drops rejected the corneal allografts at approximately 36.0 ± 3.2 days. By contrast, 8 of 10 animals given the RAPA-PGLC DDS in the anterior chamber did not reject the grafts throughout this study, which ended 90 days after surgery.

Analysis of the RI of the corneal grafts revealed a significant increase over time in the control and the PGLC carrier implantation groups, but not in the RAPA eye drop and RAPA-PGLC DDS groups (Fig. 3). On the 18th day after PK, the RI for the control and the PGLC carrier groups were 7.7 and 7.8, respectively, whereas the RI for the RAPA eye drop group was 1.9 ($P < 0.01$ compared with the control group), and the RI for the RAPA-PGLC DDS implantation group was 0.7 ($P < 0.01$). This trend continued throughout the study. By postoperative day 90, the RI for the control group was 10.4, the RI for the PGLC implantation group was 10.0, the RI for the RAPA eye drop group was 7.2 ($P < 0.01$), and the RI for the RAPA-PGLC DDS implantation group was 2.0 ($P < 0.001$).
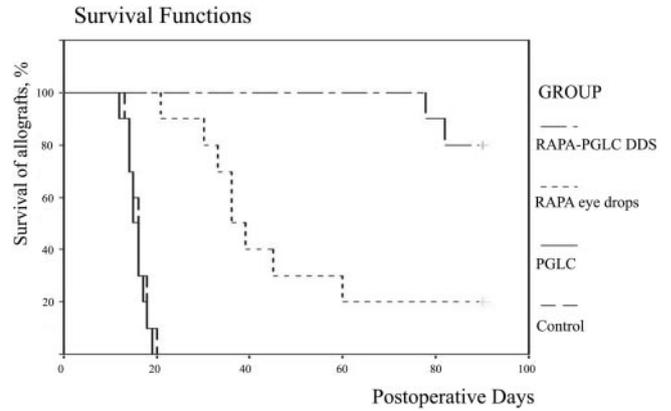

FIGURE 2. RAPA-PGLC DDS was more effective than RAPA eye drops in prolonging cornea graft survival. Allograft survival curves (Kaplan-Meier) for the control group (*dashed line*), PGLC group (*solid line*), RAPA eye drop group (*dotted line*), and RAPA-PGLC DDS group (*dashed* and *dotted lines*). Differences were statistically significant between any two groups ($P < 0.01$) except between the control group and the PGLC group ($P = 0.57$).

Effectiveness of RAPA-PGLC DDS and RAPA Eye Drops in Preventing Corneal Neovascularization

Severe vascular invasion occurred in most quadrants of the grafts in the control and PGLC carrier implantation groups. By contrast, in the RAPA eye drop and the DDS implantation groups, minor vascular invasion affecting one to two quadrants of the grafts was noted 2 weeks after PK but gradually disappeared by the 4th week. For the control, the PGLC, the RAPA eye drop, and the RAPA-PGLC DDS groups, mean neovascularization indexes were 2.4, 2.1, 0.6, and 0.3 at 2 weeks, 3.8, 3.6, 0.8, and 0.4 at 4 weeks, 3.6, 3.6, 0.6, and 0.4 at 8 weeks, and 3.8, 3.8, 0.8, and 0.4 at 12 weeks, respectively (Fig. 4).

RAPA Detected in the Aqueous Humor Only in the RAPA-PGLC DDS Group

Mean RAPA concentrations in the aqueous humor were 10.7 ± 2.5, 12.0 ± 1.8, 9.2 ± 2.3, and 7.0 ± 4.0 ng/mL in the RAPA-PGLC DDS implantation group 2, 4, 8, and 12 weeks after PK, respectively. RAPA was not detected in the RAPA eye drop group at any of these time points.
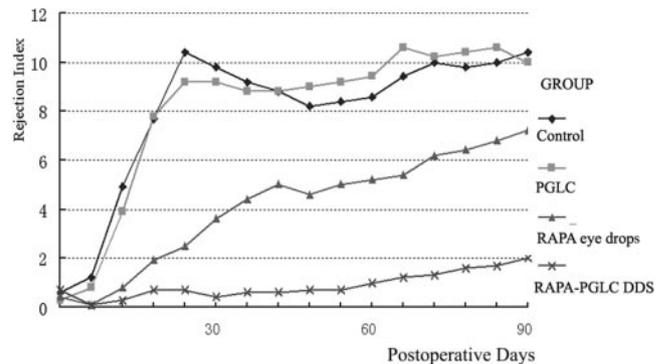

FIGURE 3. RAPA-PGLC DDS was more effective than RAPA eye drops in preventing corneal graft rejection. RI increased significantly between 1 and 4 weeks after surgery in the control and the PGLC groups and then remained at high levels. RI in the RAPA eye drop group was lower but experienced a steady increase over time. RI in the RAPA-PGLC DDS group remained low throughout the study.
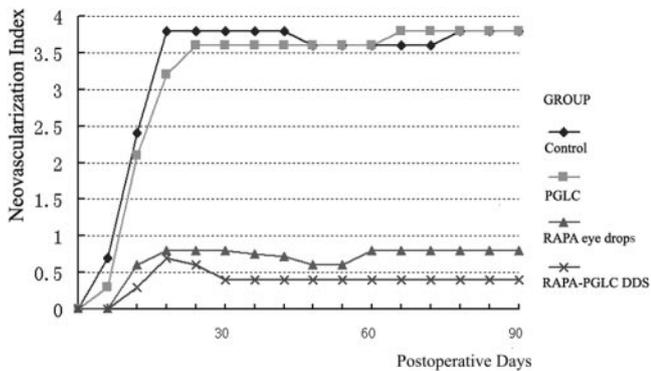
FIGURE 4. RAPA prevents neovascularization. The neovascularization index increased significantly between 1 and 3 weeks after surgery in the control and the PGLC groups and then remained at high levels. On the contrary, the neovascularization index remained low in the RAPA-treated groups. Differences between the control or PGLC group and the RAPA group were statistically significant ($P < 0.001$).

RAPA Inhibition of Expression of Proinflammatory Molecules

Edema and heavy mononuclear cell infiltration were observed in allografts in the control group and the PGLC group soon after transplantation, which led to significant increases in the thickness of the cornea. Mononuclear cell infiltration was present in all layers of the cornea; the graft margin was most heavily affected. Neovascularization was observed primarily in the stroma (Fig. 5A). The corneal graft taken at the same time point from the RAPA eye drop group had less edema and mononuclear cell infiltration. The graft taken from the RAPA-PGLC DDS group had normal corneal thickness, few infiltrating cells, and no neovascularization (Fig. 5B). Retinal, hepatic, and renal tissue sections were normal, and no inflammatory cell infiltration was observed.

In situ hybridization and immunohistochemical studies revealed significant differences among these groups. In the control and PGLC carrier–implanted groups, high levels of IL-2R, MCP-1, TNF-α, and VEGF were detected in the stroma of all corneal grafts (Figs. 5C, 5E, 5G, 5I). In the RAPA eye drop–treated group, expression of IL-2R, MCP-1, TNF-α, and VEGF was weak but detectable. By contrast, expression of these genes was not detected in the nonrejected corneal tissue of the RAPA DDS–implanted group (Figs. 5D, 5F, 5H, 5J). These results indicated that RAPA prevents corneal graft rejection by blocking proinflammatory gene expression.

DISCUSSION

Immune rejection is the major cause of human corneal graft failure. The rejection rate is approximately 20% for low-risk corneal grafts, but it is between 60% and 90% for high-risk keratoplasties with cornea vascularization.[15,16] Corneal neovascularization is the major cause of high-risk keratoplasty. Neither topical nor systemic administration of corticosteroids has been successful for prophylaxis or treatment of the rejection episode in patients at high risk. CsA is the most commonly used corticosteroid, but it does not inhibit corneal neovascular proliferation. RAPA can inhibit immune rejection of organ transplants and prevent neovascular proliferation. Therefore, it is important to determine the efficacy of RAPA in models of high-risk corneal rejection. Olsen et al.[17] first reported the usefulness of RAPA in preventing corneal graft rejection in a rat model. Their treatment protocol relied on intramuscular injections. Sundmacher's group[18] has used RAD (a novel macrocy-

clic lactone immunosuppressant derived from rapamycin) and CsA to prevent corneal immune rejection in rats. The RAD 1.5 mg/kg group was similar to the CsA 10 mg/kg group, with mean graft survival times of 37.7 and 39.7 days, respectively. The authors suggest that systemic RAPA can prolong corneal graft survival, though to a small degree, with the mean survival time increased only by 1 week, possibly because of two limitations: (1) RAPA is an oil-soluble drug that is not stable in aqueous solution, and (2) systemic administration may not
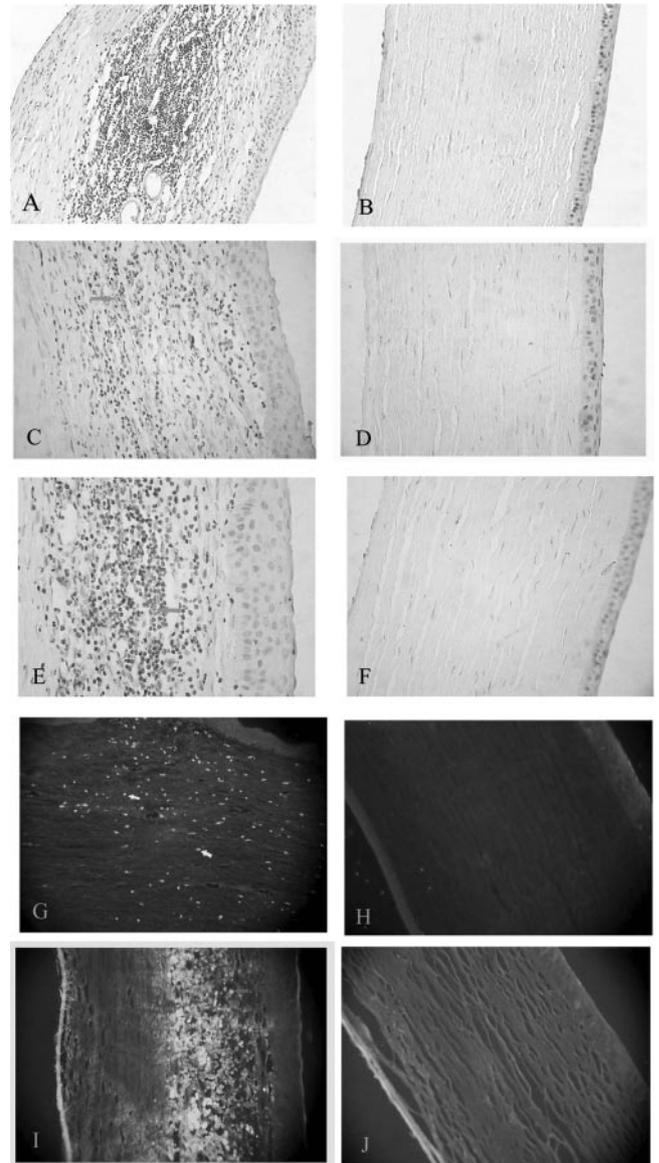

FIGURE 5. Histologic profiles of the corneal grafts. Allografts were collected on day 20. (**A–B**) Corneal graft sections of control (**A**) and RAPA-PGLC DDS (**B**) groups were stained with hematoxylin and eosin. Marked corneal thickening, inflammatory cell infiltration, and neovascularization were observed in the control group. RAPA-PGLC DDS–treated group showed normal corneal thickness and almost complete absence of inflammatory cells and neovascular vessels. (**C–F**) Gene expression in the corneal grafts detected by in situ hybridization. (**C**) IL-2R– and (**E**) MCP-1–expressing cells were detected in the control group; by contrast, no (**D**) IL-2R– or (**F**) MCP-1–expressing cells were detected in the RAPA-PGLC DDS group. (**G–J**) Protein expression in the corneal grafts detected by immunohistochemistry. High levels of TNF-α (**G**) and VEGF (**I**) proteins were detected in the control group; by contrast, little VEGF (**H**) or TNF-α (**J**) was detected in the RAPA-PGLC DDS group. Original magnifications for all panels, $\times 200$.

generate an effective RAPA concentration in the eye because of the blood-ocular barrier. To circumvent these problems, we have developed a RAPA-PGLC DDS that effectively prevents high-risk corneal allograft rejection and neovascularization. Implantable DDS can penetrate the blood-ocular barrier and confer high RAPA concentrations in the aqueous humor and, therefore, may be best suited for preventing rejection of the transplanted cornea.

However, to what degree high concentrations of RAPA in the aqueous humor are essential for preventing immune graft rejection is unclear. Similarly, it is unknown how long the drug must be present to protect the corneal allograft. After systematic administration, RAPA concentrations greater than 15 ng/mL appeared to be associated with greater risk for thrombocytopenia and hyperlipidemia, whereas RAPA concentrations lower than 6 ng/mL were associated with increased incidence of acute rejection.[19] It is also reported that the rate of acute rejection increased when everolimus (a chemical derivative of RAPA with similar activities) levels were lower than 3 ng/mL.[20] Therefore, to prevent rejection and to minimize toxicity, a therapeutic range of 4 to 12 ng/mL is recommended when sirolimus is used in conjunction with CsA. If CsA therapy is discontinued, a target range of 12 to 20 ng/mL is recommended.[21] In vitro, the IC_{50} (50% inhibitory concentration) values for peripheral blood mononuclear cells (PBMCs) of pigs and humans are 2.1 and 0.3 ng/mL for RAPA, respectively.[22] Concentrations of RAPA in aqueous humor were maintained between 7 and 12 ng/mL in the RAPA-PGLC DDS implantation group, but RAPA was not detected in the eye drop group in our study. In addition, immune rejection was observed in two rabbits in the RAPA-PGLC DDS group; RAPA was not detected in the aqueous humor of these rabbits though RAPA-PGLC DDS was seen in the anterior chamber. Therefore, it may be inferred that a high aqueous humor concentration of RAPA is extremely important for preventing high-risk corneal allograft rejection. Lack of detectable RAPA in the aqueous humor may be caused by inadequate diffusion or increased absorption of the drug. Xie et al.[23] reached similar conclusions after studying the effect of CsA in cornea transplantation in rats: the rejection episode occurs when the immunosuppressive drug concentration in the aqueous humor is reduced.

In the corneal and the conjunctival epithelia, the intercellular space is sealed by junctional complexes that prevent the transport of molecules. Corneal permeability for polyethylene glycol decreases with the increase of its molecular weight. The larger the molecular weight, the slower the permeabilities of the cornea, especially for molecules weighing more than 500 Da.[24] Therefore, it is difficult for RAPA, with a molecular weight of 914 Da, to pass through the corneal barrier. This may explain why RAPA was undetectable in the aqueous humor in the eye drop group. In eyes with implanted RAPA-PGLC DDS, RAPA was released into the aqueous humor with the continuing biodegradation of PGLC, which made it possible to maintain an effective concentration.

In our study, a small amount of RAPA was effective at inhibiting immune rejection in a rabbit model of high-risk corneal transplantation, suggesting that RAPA is a strong and effective immunosuppressive agent. The mechanism of RAPA action is distinct from that of CsA and FK506.[25] CsA and FK506 inhibit T cell proliferation from the G_0 to the G_1 phase, whereas RAPA inhibits T cell proliferation from the G_1 to the S phase. Because the G_1/S phase is one of the central "checkpoints" in the cell cycle,[26] RAPA is stronger than CsA and FK506 at suppressing T cell proliferation. Furthermore, RAPA is equally effective at inhibiting the proliferation of activated T cells. The immunosuppressive capabilities of RAPA eye drops and DDS were confirmed in our experiments. Compared with the control and PGLC–implanted groups, the RAPA eye drop–

and DDS treated–groups achieved significantly reduced levels of inflammatory gene expression, including IL-2R, MCP-1, TNF-α, and VEGF. IL-2 is the key factor to drive T cells from the G_1 to the S phase. CsA and FK506 decrease T cell proliferation by inhibiting IL-2 expression. RAPA may or may not be able to inhibit IL-2 expression, but it can block the IL-2 effect because it inhibits IL-2R expression. In addition to IL-2, TNF-α plays an important role in corneal transplantation. It can increase major histocompatibility complex II antigen expression, activate macrophages and T lymphocytes leading to more cytokine release, and cause immune rejection. RAPA reduces the expression of TNF-α, which may help prevent immune rejection.

The avascular nature of the cornea is crucial for maintaining its immune-privileged status. RAPA suppresses the growth of the neovasculature and plays an important role in reducing immune rejection after corneal transplantation. Angiogenesis is a complex process that includes the activation, proliferation, and migration of endothelial cells, the disruption of vascular basal membrane, and the formation of vascular tubes and networks, and it connects new and preexisting vascular networks.[27] Many cytokines participate in this process, including VEGF and fibroblast growth factor. VEGF appears to be the most prominent angiogenic factor. Inhibition of VEGF activity is highly effective for suppressing angiogenesis. Previous studies show that RAPA inhibits tumor neovascular growth by suppressing VEGF expression.[28] The latest investigations have found that RAPA can suppress corneal neovascularization in the alkaline-burned eye possibly by inhibiting VEGF.[29] Our data also indicated that RAPA can suppress the expression of VEGF in the cornea, which may in turn be responsible for the dramatic ablation of angiogenesis in RAPA-treated corneas.

Moreover, because the systematic administration of RAPA in humans can cause asthenia, headache, epistaxis, diarrhea, thrombocytopenia, and leukopenia after solid organ transplantation,[30] local administration of RAPA would be more advantageous. The PGLC used in this study is nontoxic and biodegradable in the human body.[31] No toxic effects attributable to RAPA-PGLC DDS were observed except the transient anterior chamber inflammatory reaction that occurred immediately after surgery and that resolved spontaneously within 3 days. Future investigations will focus on release kinetics of the drug from the polymer and modifications to the delivery system so as to achieve high steady state levels of the drug in allograft recipients.

In summary, we have found that RAPA-PGLC DDS is an effective means for delivering high concentrations of RAPA to the aqueous humor and that the PGLC-based delivery system is nontoxic in rabbits. We also showed that RAPA improved graft survival by its direct effect on inflammatory cells and angiogenesis. The former effect may play a major role in preventing graft rejection, whereas the latter effect may play an auxiliary role. Further investigations are needed to determine whether additional factors are involved in this process.

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