Cyclosporine A-loaded drug delivery systems inhibit scar formation after glaucoma surgery in rabbits

Zhao-Xing Dai[1], Xiao-Le Song[2], Xiao-Bo Yu[1], Jian-Guo Sun[3,4], Xing-Huai Sun[1,4,5]

[1]Department of Ophthalmology and Visual Science, Eye and ENT Hospital, Shanghai Medical College, Fudan University, Shanghai 200031, China;
[2]Department of Ear-Nose-Throat, Eye and ENT Hospital, Shanghai Medical College, Fudan University, Shanghai 200031, China;
[3]Research Center, Eye and ENT Hospital, Shanghai Medical College, Fudan University, Shanghai 200031, China;
[4]Key Laboratory of Myopia, NHFPC, and Shanghai Key Laboratory of Visual Impairment and Restoration, Fudan University, Shanghai 200031, China;
[5]State Key Laboratory of Medical Neurobiology, Institutes of Brain Science and Collaborative Innovation Center for Brain Science, Fudan University, Shanghai 200032, China.

To the Editor: Glaucoma is the primary cause of irreversible blindness. Surgery is the main treatment option if intraocular pressure (IOP) cannot be controlled with medications. Local fibroblast proliferation and collagen deposition cause stenosis of drainage channels and scar formation in the filtration area, which are the main causes of unsuccessful antiglaucoma surgery.[1,2] Application of mitomycin C during surgery is one of the most widely performed clinical interventions aimed at modifying the wound healing process. However, recent reports suggest that there is scope to further improve the outcomes of mitomycin C application in surgery.[3,4] Cyclosporine (CsA), a drug with low toxicity, exerts anti-inflammatory and immunosuppressive effect and has been used to suppress scar formation after filtration surgery.[5,6]

It is believed that scar formation is highly active for about 3 months after surgery, and is greatest in the first month. Thus, sustained delivery of drugs aimed at preventing scar formation could help regulate the wound healing process more effectively. We have already developed two drug delivery systems (DDS) that are able to maintain local drug concentrations at relatively stable levels, namely poly(DL-lactic acid-co-glycolic acid)-poly(ethylene glycol)-poly(DL-lactic acid-co-glycolic acid) (PLGA-PEG-PLGA) thermogel and poly(lactic-co-glycolic acid) (PLGA) coating.[7,8] In this study, we examined the effects of loading these systems with CsA on scar formation *in vivo*.

Glaucoma drainage devices (GDDs) with or without CsA-loaded DDS were implanted into both eyes of nine New Zealand rabbits. The rabbits were purchased from Yingen Rabbit House (Shanghai, China). The rabbits were housed in the animal facilities of the Eye and ENT Hospital (Fudan University) under natural light conditions with free access to food and water. All experimental protocols, including the experiments, transportation, and care of the animals, complied with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research and the guidelines of the Animal Care and Use Committee of Fudan University (Shanghai, China). The eyes were randomized into a coat group, a gel group, and a control group. All the procedures were completed by the same ophthalmologist. The evaluator was blinded to the allocated groups during the assessments.

To increase the gel's visibility, we mixed DiI, a lipophilic orange-red fluorescent dye, into the drug-loaded gel. Every 7 days after surgery, IOP was measured with a Tono-pen (Tono-Pen AVIA Tonometer; Reichert Technologies, Depew, NY, USA). Bleb morphology was analyzed by an imaging system mounted to a digital camera (EOS 60D; Canon Inc., Tokyo, Japan) and a slit lamp (YZ5J; 66 VisionTech, Suzhou, China). During the follow-up, we focused on side effects such as bleb leakage, conjunctival edema, hyphemia, shallow anterior chamber (degree I–III), and infection.

We used the Indian Bleb Appearance Grading Scale (IBAGS) to assess bleb morphology[9] and calculated mean IBAGS scores for each group at each time point. The IBAGS scores were compared by two-way repeated-measures analysis of variance. Dunnett's T3 *post hoc* test was used for multiple comparisons. A value of $P < 0.05$ was considered statistically significant.

Anterior chamber radiography was performed 4 weeks after surgery. During anterior chamber radiography, eyes were injected with 100 μL of 1% fluorescein (Alcon Laboratories, Inc., Fort Worth, TX, USA) and observed under cobalt blue light to determine whether the filtration

Access this article online

Quick Response Code:

Website: www.cmj.org

DOI: 10.1097/CM9.0000000000000234

Correspondence to: Prof. Xing-Huai Sun, Department of Ophthalmology, Eye and ENT Hospital of Fudan University, 83 Fenyang Road, Shanghai 200031, China
E-Mail: xhsun@shmu.edu.cn

Chinese Medical Journal 2019;132(11)

Received: 26-12-2018 **Edited by:** Yuan-Yuan Ji

channels were open or closed. The number of open pathways was recorded for all eyes in each group. We also assessed whether the filtration pathway was partly or completely open based on whether the diffusion area was smaller than or equal to the bleb range.

All rabbits were sacrificed 12 weeks after surgery. All eyes were removed and fixed in 4% paraformaldehyde. The tissue samples were embedded in paraffin, sectioned through the minor axis of the GDD body, and stained with hematoxylin and eosin (H&E), and photographs were taken under a microscope (LEICA DM 4000B; Leica Microsystems Inc., Buffalo Grove, USA). Then, the photographs of each tissue section from individual eyes were merged using Adobe Photoshop CC (Ver 14.0). The morphology of each bleb was qualitatively evaluated and compared among the three groups.

After GDD implantation, we found no degree II or III shallow anterior chambers, drainage tube occlusion, choroidal detachment, GDD exposure, corneal endothelial decompensation, cataract, vitreous hemorrhage, or conjunctival erosion in any eye.

The mean IOP in each group is shown in Figure 1A, and logarithmic fitting curves revealed some differences between each group. Of note, the IOP in all three groups decreased significantly within 7 days after surgery compared with before surgery, with P values of 0.047, 0.021, and <0.001 for the control, gel, and coat groups, respectively. Compared with the control group, there was a significant decrease in IOP in the coat group ($P < 0.001$) but not in the gel group ($P = 0.345$). Therefore, these results suggest that CsA significantly decreased postoperative IOP, especially when applied as a coat on the GDD, compared with the control group.

Bleb morphology was assessed under both diffuse and slit light [Figure 1B]. In the diffuse light condition, the morphology of the blebs could be seen clearly, together with wound healing and conjunctiva transparency. Under a slit lamp, we determined the relationships between the cornea and iris, and that between the conjunctiva and GDD body or sclera. In all three groups, we could see that the blebs uplifted with rapid congestion, which quickly reached the maximum within 7 days after surgery. The blebs then gradually flattened and congestion gradually decreased. In the early stage, especially within 7 days after surgeries, bleb morphology was not markedly different among the three groups. However, in the late stage, the blebs gradually flattened with different rates among the three groups.

At 7 days after surgery, the height of the filtering blebs had begun to decrease in the coat and the control groups [Figure 1D1]. The height of blebs decreased at a significantly slower rate in the gel group ($P < 0.001$), which is partly due to the mass effect of the gel. The area of the blebs in both CsA groups was greater than that that in the control group ($P < 0.001$, Figure 1D2). The vascularization score was significantly lower in the coat group than in the control group ($P = 0.01$) but was not significantly different between the gel and the control groups [Figure 1D3].

Anterior chamber radiography was performed for all eyes 4 weeks after surgery. Figure 1C shows representative images of these processes. The resulting images showed that the proportion of open drainage pathways was greater in the gel and the coat groups than that in the control group. In the control group, only three eyes (the first, fifth, and sixth image of the control group in Figure 1C) had open pathways, whereas all pathways were open in all eyes in both the CsA groups. The diffuse area of fluorescence was also very limited in the three open pathways in the control group. In contrast, the diffusion areas were significantly larger in both the CsA groups, and only two blebs in each group showed limited diffusion (the first, third image of the coat group and the third, fourth image of the gel group in Figure 1C). Radiography revealed that the fluorescent region in the gel group was covered by the gel, but the drainage pathways and diffuse area of fluorescence were clearly visible. This direct evidence demonstrates that administration of CsA improved the opening of drainage pathways regardless of the DDS used.

After sacrificing the rabbits, their eyes were trimmed and embedded in paraffin, and sections were stained with H&E. We assessed bleb morphology in each group, and sections are shown in Figure 1E. The key finding of H&E staining was that there was less scar tissue in the gel and the coat groups than that in the control group, indicating that the CsA inhibited scar tissue formation in rabbit eyes. Moreover, the blebs were significantly larger in the gel and the coat groups than those in the control group. The smaller blebs in the control group might be due to contraction of scar tissue, providing further evidence of the anti-scar effects of CsA.

The main objective of glaucoma surgery is to slow or prevent further damage of the retinal nerve fiber by controlling IOP. Postoperative IOP is an important index for the prognosis of glaucoma surgery. In this study, both preoperative and postoperative IOPs were followed up to indicate the effect of novel GDDs on humor aqueous drainage, showing a positive effect on the drainage of the novel GDD.

To evaluate the function of filtering blebs after glaucoma surgeries, we employed both direct and indirect methods. For the direct method, fluorescent dye was injected into anterior chamber to visualize how the aqueous humor was drained through the tubes and into the blebs. For indirect methods, the morphology of blebs and IOP were measured to reflect the filtrating function.

The mass effect of the gel played an important role in affecting the morphology of blebs. As the gel existed in the blebs for more than 3 months, it always kept the height of the blebs. Additionally, the red-orange dye in the gel affected the observation of the vascular to some extent because of the similar color. It is reasonable to conjecture that the vascularization might be overestimated in the gel group. So, the difference between the coat and the control group reflected the effect of CsA on vascularization better.

In this study, we applied two DDSs, PLGA-PEG-PLGA thermogel and PLGA coat, to continuously delivery CsA

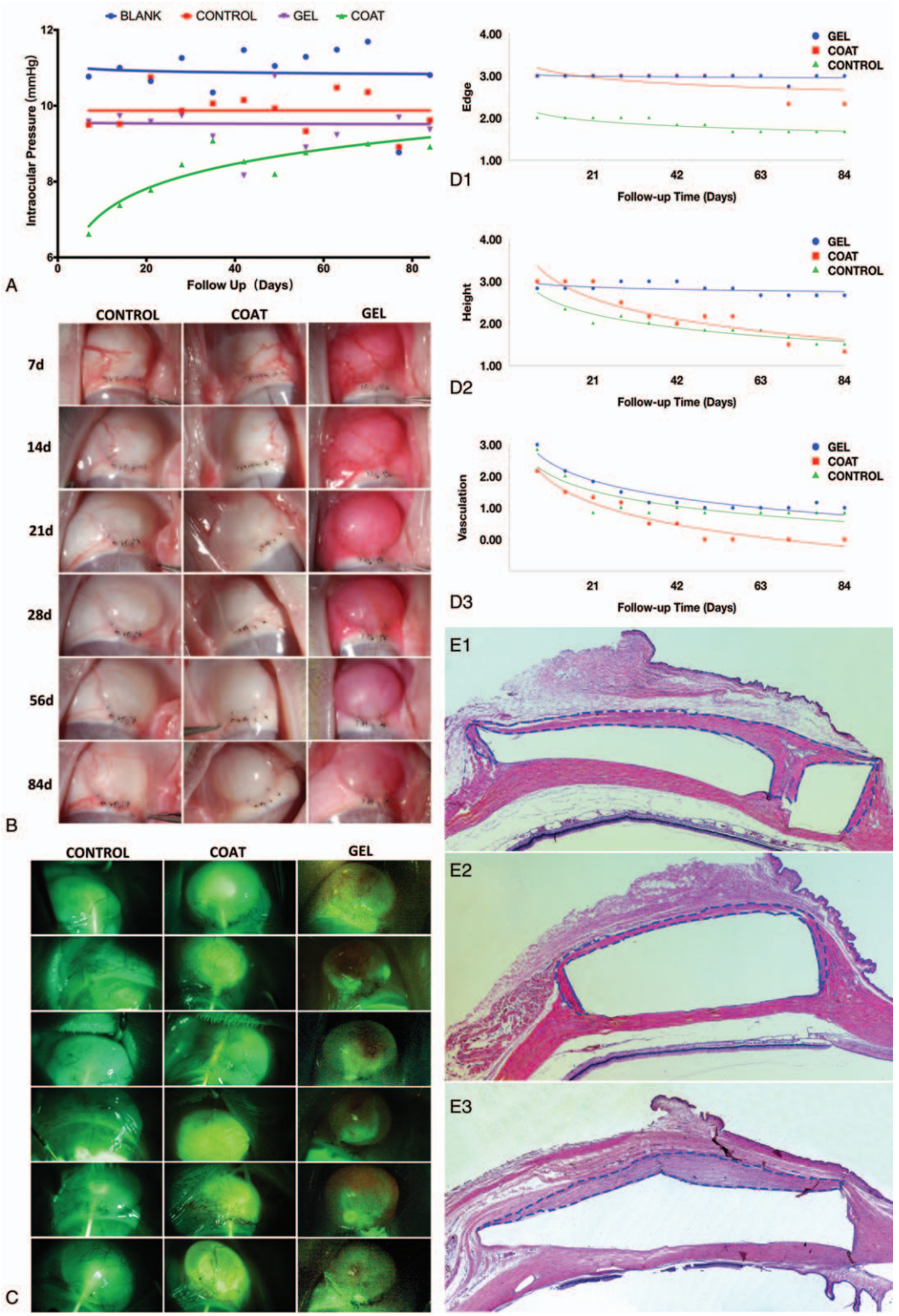

Figure 1: Results of *in vivo* experiments. (A) Mean intraocular pressure in all three groups was fitted onto logarithmic curves. Compared with the control group, IOP was significantly lower in the coat group ($P < 0.001$) but not in the gel group ($P = 0.345$). (B) Changes in bleb morphology over time. (C) Anterior chamber radiographs taken 30 days after surgery. The images were taken once the fluorescence had grown to the largest area. (D) Quantification of bleb morphology in terms of bleb height (D1), width (D2), and vascularization (D3). Bleb morphology was assessed according to the Indiana Bleb Appearance Grading Scale classification. (E) Histological analysis of the surgical area of an eye in the control group (E1), coat group (E2), and gel group (E3). The blue dashed lines indicate scar tissue in the surgical area (H&E staining, original magnification ×100). H&E: Hematoxylin and eosin; IOP: Intraocular pressure; 1 mmHg = 0.133 kPa.

into the surgery region. Their main components are both high molecular polymers, the ratio of which was adjusted to achieve different properties and stable delivery of drug. Through these two DDSs, we demonstrated the *in vivo* effect of CsA. The coat worked better in lowering the IOP, whereas the gel group had better bleb morphology. They both significantly improved the anterior chamber radiography.

In conclusion, this study suggests that CsA is a promising drug for preventing scar tissue formation after glaucoma surgery. Loading CsA into two different types of GDD (coat and gel) proves to be effective in improving the prognosis of antiglaucoma surgery.

Funding

This study was supported by a grant of Shanghai Natural Science Foundation (No. 15ZR1405900).

Conflicts of interest

None.

References

1. Sommer A. Intraocular pressure and glaucoma. Am J Ophthalmol 1989;107:186–188. doi: 10.1016/0002-9394(89)90221-3.
2. Sherwood MB. A sequential, multiple-treatment, targeted approach to reduce wound healing and failure of glaucoma filtration surgery in a rabbit model (an American Ophthalmological Society thesis). Trans Am Ophthalmol Soc 2006;104:478–492. doi: 10.1016/S0002-9394(20)90304-5.
3. Mesci C, Erbil HH, Karakurt Y, Akcakaya AA. Deep sclerectomy augmented with combination of absorbable biosynthetic sodium hyaluronate scleral implant and mitomycin C or with mitomycin C versus trabeculectomy: long-term results. Clin Exp Ophthalmol 2012;40:e197–e207. doi: 10.1111/j.1442-9071.2011.02611.x.
4. Law SK, Kornmann HL, Giaconi JA, Kwong A, Tran E, Caprioli J. Early aqueous suppressant therapy on hypertensive phase following glaucoma drainage device procedure: a randomized prospective trial. J Glaucoma 2016;25:248–257. doi: 10.1097/IJG.0000000000000131.
5. Akyol N, Demir T, Cobanoglu B, Ulas F. Ciliary body toxicity of cyclosporin A and octreotide acetate in rabbit eyes: a comparison with mitomycin C and 5-fluorouracil. Graefes Arch Clin Exp Ophthalmol 2003;241:816–822. doi: 10.1007/s00417-003-0717-6.
6. Turaçli ME, Gündüz K, Aktan G, Sencer H. Topical cyclosporine as a possible new antimetabolite in trabeculectomy. Ophthalmic Surg Lasers 1996;27:438–444. doi: 10.3928/15428877-20110420-01.
7. Dai Z, Yu X, Hong J, Liu X, Sun J, Sun X. Development of a novel CsA-PLGA drug delivery system based on a glaucoma drainage device for the prevention of postoperative fibrosis. Mater Sci Eng C Mater Biol Appl 2016;66:206–214. doi: 10.1016/j.msec.2016.04.077.
8. Sun J, Lei Y, Dai Z, Liu X, Huang T, Wu J, *et al*. Sustained release of brimonidine from a new composite drug delivery system for treatment of glaucoma. ACS Appl Mater Interfaces 2017;9:7990–7999. doi: 10.1021/acsami.6b16509.
9. Picht G, Grehn F. Classification of filtering blebs in trabeculectomy: biomicroscopy and functionality. Curr Opin Ophthalmol 1998;9:2–8. doi: 10.1097/00055735-199804000-00002.

How to cite this article: Dai ZX, Song XL, Yu XB, Sun JG, Sun XH. Cyclosporine A-loaded drug delivery systems inhibit scar formation after glaucoma surgery in rabbits. Chin Med J 2019;132:1381–1384. doi: 10.1097/CM9.0000000000000234