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*Original Article*

# Intraoperative intravitreal injection of dexamethasone PLGA nanoparticle inhibited expression of aqueous humor TGF-β2 and MMPs in cataract patients

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**Abstract:** To investigate the inhibitory effect of intraoperative intravitreal injection of dexamethasone polylactic-co-glycolic acid (PLGA) nanoparticle on the e expression of aqueous humor TGF-β2 and MMPs. 70 cases of cataract patient (106 cases of affected eyes) were randomized into six groups, including sham group, regular dexamethasone group, PLGA nanoparticle control group, 100 μg dexamethasone PLGA nanoparticle group, 200 μg dexamethasone PLGA nanoparticle group, and 400 μg dexamethasone PLGA nanoparticle group. Different suspension was injected for each group accordingly. Drug releasing time, formation rate of choroidal neovascularization (CNV), and postoperative expression of aqueous humor TGF-β2 and MMPs (MMP-2 and MMP-9) were examined, respectively. Compared with regular dexamethasone treatment, dexamethasone PLGA nanoparticle treatment significantly prolonged drug releasing time, with a minimum of 56 days. The same phenomenon was observed in PLGA nanoparticle control group that a small amount of residual drug particles still existed in inferior vitreous cavity 56 days after photocoagulation. Drug releasing time of regular dexamethasone group was short with an average of 14 days. Compared with sham group and PLGA nanoparticle control group, all dexamethasone PLGA nanoparticle groups had a significantly lower CNV formation rate and decreased expression of aqueous humor TGF-β2, MMP-2 and MMP-9 ($P<0.05$). Intraoperative intravitreal injection of dexamethasone PLGA nanoparticle had a promising inhibitory effect on aqueous humor TGF-β2 and MMPs for cataract treatment.

**Keywords:** Dexamethasone, PLGA, nanoparticle, vitreous, cataract

## Introduction

Cataract, characterized by multiple symptoms including impaired vision, double vision, muscae volitantes and nyctalopia, is caused by lens denaturation and turbidity via metabolic disorders. Cataract is the leading cause of blindness worldwide [1]. With the increase of aged population in recent years, the incidence of cataract gets higher year by year in China [2]. Statistics showed that cataract accounts for 41.06% of blindness and 49.38% of low vision [3]. Current optimal therapeutic regimen for cataract is operation removal, of which the most used strategy was phacoemulsification combined with intraocular lens implantation. Despite of advantages such as small incision, rapid healing and promising vision recovery, postoperative limitations of surgery includes vitreous hemorrhage, retinal detachment and recurrence [4]. Studies proved that choroidal neovascularization angiogenesis was the most common pathologic change in late stage of fundus lesions [5]. Glucocorticoid was a commonly used drug to inhibit proliferative viteoretinopathy in ophthalmology. However, because of systemic side effect and specific ocular physiologic structure, many physicians recommended vitreous intraocular administration rather than intravenous administration or oral administration. Yasukawa T etc. found vitreous intraocular single-administration was characterized with rapid drug elimination, short lasting effect and high incidence of complications. Nanoparticle is a novel sustained release medication system with biodegradability which controls drug con-

centration within certain range and keeps long-term drug sustained release to improve efficacy [6]. To improve cataract's postoperative efficacy and prognosis, 70 cases of cataract patient were included into our research, provided intraoperative intravitreal injection of dexamethasone PLGA nanoparticle and postoperative expression of aqueous humor TGF-β2 and MMPs was measured.

## Materials and methods

### *Objects of study*

70 cases cataract patients with an average age of 39.67±12.57 (range of 26-69) who received treatment between September 2013 and September 2015 in Linyi people's hospital of fraternity eye clinic were included. There are 37 males (56 eyes) and 33 females (50 eyes). The patients were randomly divided into 6 groups: 100 μg dexamethasone PLGA nanoparticle, 200 μg dexamethasone PLGA nanoparticle, 400 μg dexamethasone PLGA nanoparticle (10 cases each group), control nanoparticle (10 cases), dexamethasone (10 cases) and normal saline (15 cases) group. There was no statistical difference (P>0.05) between the basic situation of patients mentioned above.

The study protocol was approved by the Research Ethics Committee of Linyi people's hospital, and all patients were given informed consent before study commencement.

### *Inclusive criteria*

1) Patients were diagnosed with cataract. 2) No other serious eye diseases, such as retinal detachment, macular retinoschisis, or posterior uveitis; 3) No injection of dexamethasone PLGA nanoparticle to the vitreum of eyes [7].

### *Exclusion criteria*

1) Patients have eye diseases and other diseases, or eye surgery have been performed. 2) Patients have other primary diseases such as heart diseases, brain diseases, kidney diseases and so on. 3) Patients have been treated with glucocorticoid drugs.

### *Regents and instruments*

Dexamethasone was provided by Tianjin pharmaceutical group company; Rabbit anti VIII factor polyclonal antibody, Immunohistochemical SP, and AEC kit were purchased from Wuhan Boster bioengineer company; Silt lamp bio microscope, confocal laser fundus angiography, and visual electrophysiology detector were purchased from Pinyin Baobao technological company.

### *Methods*

1) Treatment method: drugs of each group mentioned above were prepared with sterile saline, 10 μL dexamethasone suspension, 10 μL control PLGA nanoparticle suspension, or 10 μL dexamethasone suspension was injected into vitreum of corresponding groups. 2) Internal injection: after photocoagulation, affected eyes were anaesthetized by 1% tetracaine hydrochloride, sclera wall was exposed after separating the bulbar conjunctiva. An incision of 0.5 mm in the sclera was performed by syringe needle, and the drug was injected into the vitreous chamber through the incision [8].

### *Observation*

Vitreum changes were measured by flash electroretinogram at day 7, 14, 28, 56 to evaluate the function of retina. The fade time of drug in vitreum was observed in the corresponding time point after photocoagulation. Choroid neovascularization (CNV) was observed by fluorescein fundus angiography at day 14, 21, 56 after photocoagulation, rate of CNV were estimated by the leakage of fluorescence in fluorescein angiography [9].

### *TGF-β2 in aqueous humor of patients*

After treatment, the expression of MMPs (MMP-2, MMP-9) in membranes was detected by immunohistochemistry. Criteria for protein expression [10]: brown granules displayed in cytosol or cytoplasm represented positive expression. The expression was then scored based on cytoplasmic staining intensity and the proportion of positive cell. Scoring criteria were as follows [11]: 1) staining intensity: almost no staining for 0, fallow for 1, brown for 2, very brown or golden yellow for 3; 2) positive cell rate: no positive cells for 0, positive cells less than 50% for 1,positive cells between 50%-75% for 3, over 76% for 3; Negative (-) represented the sum of both between 0-1, weak positive (+) represented 2-3 scores, positive (++) represented 4-6 scores.

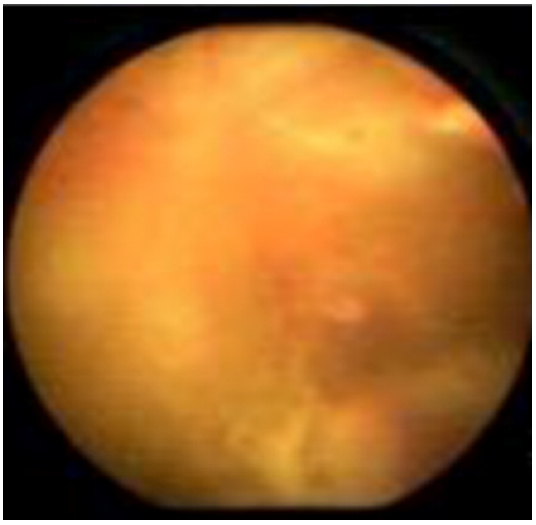

**Figure 1.** Vitreous opacity and residual drug particles were observed in regular dexamethasone group in 1-day after photocoagulation. Laser photocoagulation sign was seen indistinctly.

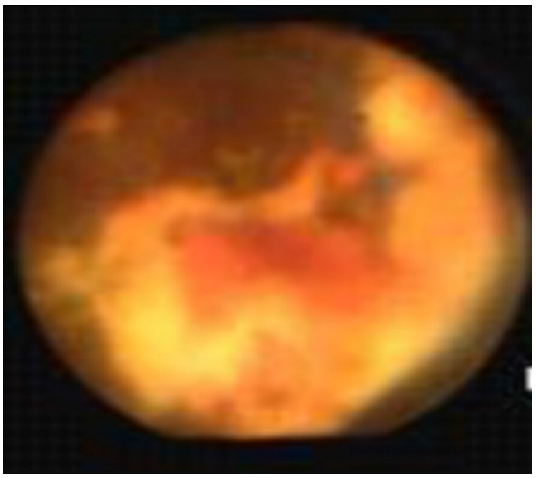

**Figure 2.** Vitreous opacity and residual drug particles were significantly observed in 200 μg dexamethasone PLGA nanoparticle group in 1-day after photocoagulation. Laser photocoagulation sign was dim.

*Statistical analysis*

Results of this study were summarized and analyzed by SPSS 16.0 software, enumeration data showed as percentage t-test was used for comparison among groups, normal distribution measurement displayed as (±s). *P* value <0.05 was considered to be statistically significant.

## Results

*Postoperative vitreous change*

Marked vitreous opacity was identified in all dexamethasone PLGA nanoparticle group, PLGA nanoparticle control group and regular dexamethasone group 1-day after photocoagulation (**Figures 1**, **2**), and dexamethasone PLGA nanoparticle had a longer drug releasing time in vitreous body and chorioido-retinal tissue with a minimum of 56 days. Nanoparticle degraded slowly in PLGA nanoparticle control group and a small amount of residual drug particles still existed in inferior vitreous cavity 56 days after photocoagulation. Regular dexamethasone group was characterized with short releasing time with an average of 14 days. The drug was eliminated rapidly and absorbed in 14-day after photocoagulation (**Figure 3**).

*Comparison of CNV formation rates*

Compared with sham group and PLGA nanoparticle control group, CNV formation rate was significantly lower in other dexamethasone groups, including regular dexamethasone group, PLGA nanoparticle control group, 100 μg dexamethasone PLGA nanoparticle group, 200 μg dexamethasone PLGA nanoparticle group and 400 μg dexamethasone PLGA nanoparticle group, at 14-day, 21-day and 56-day after photocoagulation, respectively (P<0.05). Compared with 100 μg dexamethasone PLGA nanoparticle group and 200 μg dexamethasone PLGA nanoparticle group, CNV formation rate in 400 μg dexamethasone PLGA nanoparticle group was lower at 14-day, 21-day after photocoagulation (P<0.05). CNV occurred in regular dexamethasone group at 56-day after photocoagulation. Data was shown in **Table 1**.

*Postoperative expression of TGF-β2 in aqueous tumour*

Compared with sham group and PLGA nanoparticle control group, postoperative expression of TGF-β2 in aqueous humour was significantly lower in other dexamethasone groups (P<0.05), Data was shown in **Table 2**.

*Expression of MMP-2 and MMP-9 in PVR membranes*

Compared with sham group and PLGA nanoparticle control group, positive expression rates of MMP-2 and MMP-9 were significantly lower in other dexamethasone groups (P<0.05). Compared with 100 μg dexamethasone PLGA nanoparticle group, positive expression rates of

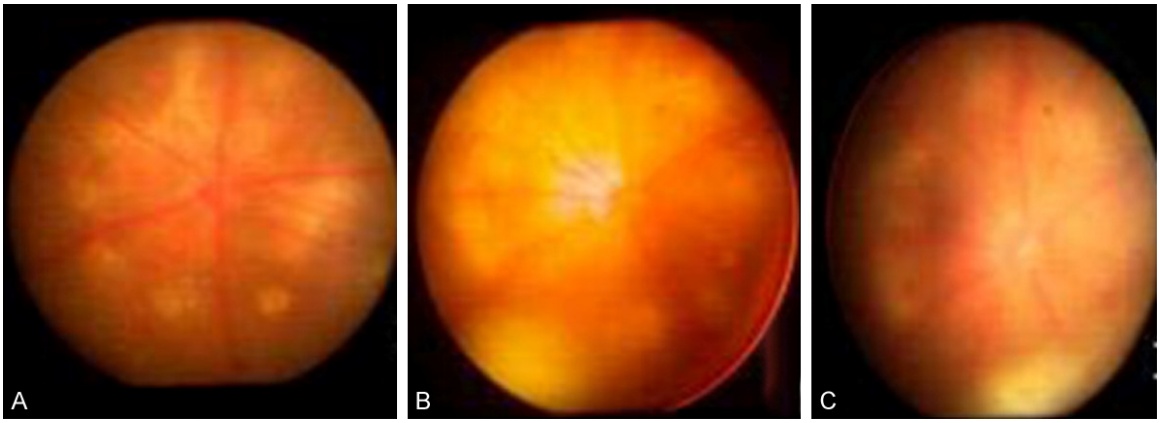


**Figure 3.** Post-photocoagulation drug absorption in three groups. A. Drug was absorbed completely in regular dexamethasone group 14 days after photocoagulation. B. 200 μg dexamethasone PLGA nanoparticle group 14 days after photocoagulation. C. Laser photocoagulation and residual drug particles were seen in 200 μg dexamethasone PLGA nanoparticle group 56 days after photocoagulation.

**Table 1.** Comparison of CNV formation rate (%)

| Group | Sample (N) | CNV formation rate (%) | | |
|---|---|---|---|---|
| | | 14-day | 21-day | 56-day |
| 100 μg dexamethasone PLGA nanoparticle group | 10 | 27.3 | 26.8 | 24.7 |
| 200 μg dexamethasone PLGA nanoparticle group | 10 | 15.9 | 15.1 | 14.5 |
| 400 μg dexamethasone PLGA nanoparticle group | 10 | 8.1 | 7.8 | 7.5 |
| Regular dexamethasone group | 10 | 32.1 | 32.5 | 35.8 |
| PLGA nanoparticle control group | 15 | 66.2 | 65.4 | 64.2 |
| Sham group | 15 | 65.9 | 65.2 | 64.1 |

**Table 2.** Comparison of postoperative expression of TGF-β2 in aqueous humour ($\bar{x}\pm s$)

| Group | Sample (N) | TGF-β2 (pg/mL) |
|---|---|---|
| 100 μg dexamethasone PLGA nanoparticle group | 10 | 463±103 |
| 200 μg dexamethasone PLGA nanoparticle group | 10 | 402±112 |
| 400 μg dexamethasone PLGA nanoparticle group | 10 | 387±83 |
| Regular dexamethasone group | 10 | 482±131 |
| PLGA nanoparticle control group | 15 | 580±142 |
| Sham group | 15 | 592±136 |

MMP-2 and MMP-9 in 400 μg dexamethasone PLGA nanoparticle group was significantly lower ($P<0.05$) (**Figure 4**).

## Discussion

Various pathogenic factors contribute to cataract. Cataract had a high incidence, and the main therapy to improve quality of patient life was phacoemulsification combined with intraocular lens implantation. Researchers found lens extraction resulted in postoperative loss of intraocular contents and decreased supporting role of crystalline lens, thereby causing lens ante-displacement and enlarged movement range of lens. Consequently, traction to surrounding tissues was increased and the risk of retinal detachment and high myopia eyes was also increased [12]. Surgical injury, deterioration of aqueous barrier, and cytokine denature led to the production of a huge amount of additional cytokines after operation [13]. Therefore, surgical treatment of cataract had some disadvantages and high incidence of postoperative complications. Main pathological change of postoperative recurrent cataract and retinal detachment is choroidal neovascularization, so inhibition of choroidal neovascularization may improve postoperative prognosis. Previous studies showed that glucocorticosteroid promisingly inhibited ocular angiogenesis [14]. Our study was

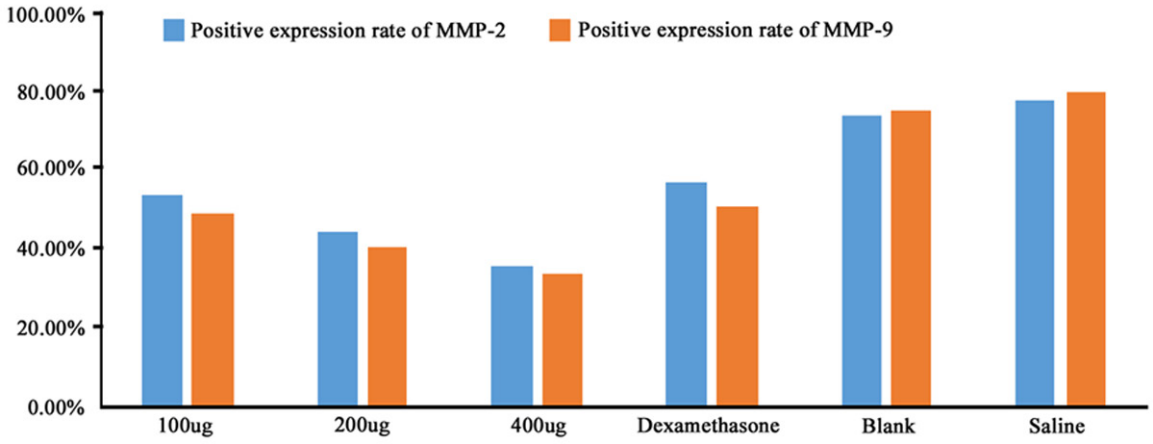


**Figure 4.** Positive expression rate of MMP-2 and MMP-9 in PVR.

focused to explore detailed effect of intraoperative intravitreal injection of dexamethasone PLGA nanoparticle on expression of TGF-β2 and MMPs.

Dexamethasone belongs to the class of glucocorticosteroid. Its pharmacological effects include anti-inflammatory, anti-immunity, antitoxin and anti-shock [15], moreover, anti-immunity and anti-inflammatory effects play pivotal roles in the inhibition of ocular angiogenesis. Detailed mechanism of Dexamethasone inhibits synthesis of DNA, RNA and proteins to repress mitosis, inflammatory response, cell proliferation and migration [16]. Harkness et al. found dexamethasone inhibited expression level and activity of TGF-β2 and MMPs in vascular endothelial cells [17]. Direct dexamethasone treatment takes effect quickly but has a short lasting time, so there is no promising prognosis in treatment of oculopathy. Polylactic-co-glycolic acid (PLGA) was widely utilized in clinical treatment with many advantages including low immune response, biodegradability, and promising biocompatibility. Therefore, PLGA is used as an adjuvant of slow-release drug [18]. PLGA not only improved the method of drug delivery, but also prolonged drug releasing time to improve efficacy and safety [19]. Nanoparticle belongs to solid colloid particles, and is being used as a promising intravenous injection drug carrier because it does not react with accompanied drug due to its specific molecular properties [20]. Hence, nanoparticle is an ideal releasing or targeting agents for injection application to maintain molecular stabilization of accompanied drug during medication [20]. We hypothesized PLGA nanoparticle could be used as excipient for dexamethasone intravitreal injection to improve its efficacy and disease prognosis. Our study showed dexamethasone PLGA nanoparticle had a longer drug releasing time with a minimum of 56 days, while regular dexamethasone treatment only hand a 14-day drug releasing time. This indicated PLGA nanoparticle in addition to dexamethasone significantly improved dexamethasone's efficacy. On the other hand, compared with control groups, CNV formation rates decreased in all dexamethasone PLGA nanoparticle groups, as well as expression of TGF-β2, MMP-2 and MMP-9 ($P<0.05$). Though our study results confirmed our hypothesis, some limitation exists in this study, for instance, among various types of matrix metalloproteinase, we only measured the expression of MMP-2 and MMP-9, the expression of other MMPs remains unclear and needs to be further explored. A larger number of samples might be helpful in future study.

In conclusion, Intraoperative intravitreal injection of dexamethasone PLGA nanoparticle inhibited postoperative expression of aqueous humor TGF-β2, MMP-2 and MMP-9 in cataract patients. This novel treatment improved cataract prognosis and is worth being widely spread.

## Disclosure of conflict of interest

None.

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