

Effects of diquafosol sodium in povidone iodine-induced dry eye model

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Abstract

• **AIM:** To investigate the effects of diquafosol sodium (DQS) for dry eye model induced with povidone-iodine (PI) solution.

• **METHODS:** Ten Sprague Dawley rats as the control group. Thirty Sprague Dawley rats were used to establish the dry eye model with stimulation of 10 g/L PI for 14d, then divided rats into three groups: dry eye group with no treatment (DED group, $n=10$); phosphate buffer saline treated group (PBS group, $n=10$); diquafosol treated group (DQS group, $n=10$). Clinical changes were observed by tear production test, fluorescein staining, tear break-up time (TBUT) test, corneal confocal microscope and ocular surface comprehensive analyzer. Eyeballs were collected on day 10 of treatment for hematoxylin-eosin (HE), periodic acid-Schiff (PAS), and alcian blue staining. TUNEL assay, polymorphonuclear (PMN) and mucin 1 (MUC1) immunofluorescence were performed and corneal ultrastructural changes were detected by electron microscopy.

• **RESULTS:** Compared with DED and PBS groups, tear production (7.26 ± 0.440 vs 4.07 ± 0.474 mm; 7.26 ± 0.440 vs 3.74 ± 0.280 mm; all $P<0.01$) and TBUT (7.37 ± 0.383 s vs 1.49 ± 0.260 s; 7.37 ± 0.383 s vs 1.42 ± 0.437 s; all $P<0.01$) were significantly increased in DQS group. HE, PAS, and alcian blue staining and MUC1 immunofluorescence showed mucins and conjunctival goblet cells density (8.45 ± 0.718 vs 5.21 ± 0.813 cells/ 0.1 mm^2 ; 8.45 ± 0.718 vs

5.36 ± 0.615 cells/ 0.1 mm^2 ; all $P<0.01$) increased in DQS group. Confocal microscopy, PMN immunofluorescence and TUNEL staining showed inflammatory infiltration and corneal epithelial cells apoptosis decreased in DQS group. The increased number of microvilli in corneal epithelial and the recovered cell junction were observed in DQS group.

• **CONCLUSION:** PI instillation can induce goblet cells and mucin loss, epithelial cell apoptosis and inflammation, which are consistent with the pathological manifestations of dry eye. Diquafosol can repair the ocular surface damage caused by PI, reduce corneal inflammation, inhibit corneal epithelial cell apoptosis, promote mucin secretion and maintain tear film stability.

• **KEYWORDS:** diquafosol; povidone iodine; dry eye; mucin; inflammation; tear film; rats

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INTRODUCTION

Dry eye disease (DED) is a heterogeneous disorder of the chronic ocular surface encompassing a range of signs and symptoms associated with impaired ocular lubrication. The abnormality in the quality, quantity, and dynamics of tear may result in tear film instability or imbalance of the ocular surface microenvironment, which may be accompanied by inflammatory reactions, ocular surface destruction, and abnormal distribution and function of the corneal nerve^[1-2].

Tear film instability is the core pathogenic mechanism of dry eye^[3], the mucin layer covers the ocular surface and sustains tear film stability. The destruction of ocular surface integrity, including goblet cell loss and apoptosis, may result in abnormality of mucins, which in turn leads to tear film instability. Tear instability is related in increased tear osmolarity, which results in ocular surface inflammation^[4-5].

Povidone-iodine (PI) is widely used for preoperative disinfection of ophthalmic surgery with a broad spectrum of microbicidal activity^[6]. Many studies established that PI was toxic to cornea, with increasing exposure time of 5% PI. Kim

et al^[7] observed pathophysiological ocular surface changes, such as loss of goblet cell density and damaged cornea epithelium, which were similar to those observed with dry eye disease.

In our previous studies, we observed ocular surface changes of Sprague Dawley rats after topical instillation of PI (1%, twice daily, 14d). Tear break-up time (TBUT; $0.87\pm 0.307s$, $8.15\pm 0.564s$; $P<0.01$) and tear production (1.47 ± 0.374 , 7.96 ± 0.422 mm; $P<0.01$) of rats in experimental group significantly decreased compared with rats in blank control group. On day 14, we collected eyeballs for hematoxylin-eosin (HE) and periodic acid-Schiff (PAS) staining, we observed loss of goblet cells and inflammation of cornea and conjunctiva in rats of experimental group. Clinical parameters and histopathological changes in rats were comparable to those with DED. In consequence, we successfully induced a dry eye model by 1% PI (Figures 1A, 2).

Diquafosol, a P2Y2 receptor agonist, could activate P2Y2 receptors in ocular surface, which exists in conjunctival epithelium, lacrimal gland and meibomian gland. It had been demonstrated to improve secretion of mucin and fluid transport, eventually maintain tear film stability^[8-10]. Many clinical trials reported encouraging clinical improvements with diquafosol in dry eye treatment, including improvements of corneal fluorescein staining score, TBUT and tear production^[11-12].

This study aimed to investigate the changes of ocular surface with PI and therapeutic effects and mechanism of diquafosol in dry eye rats.

MATERIALS AND METHODS

Ethical Approval All experiment procedures abided the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research and were ratified by the Ethics Committee of Xuzhou Medical University (Approval ID: 202110A336, Xuzhou, China).

Experiment Animals Forty female Sprague Dawley rats (160–180 g, eight weeks old, provided by Xuzhou Medical University Laboratory Animal Center, Xuzhou, China) were selected. All rats were free of ocular disease with examining the anterior segment *via* slit lamp microscope. Tear production, fluorescein staining score and TBUT were measured to confirm the rats were without DED. Rats lived in a standard environment (room temperature $25^{\circ}\text{C}\pm 1^{\circ}\text{C}$, relative humidity $60\%\pm 10\%$, alternating 12-hour light-dark cycles), ate and drank at ease.

Experimental Procedures Ten female rats were used as blank control group, and 30 female rats were used to establish dry eye model with instillation of 20 μL 10 g/L PI solution both eyes twice daily for 14d (Yuexing, Xinghua, China). Tear production, fluorescein staining score and TBUT were

measured to confirm the 30 rats were successfully induced DED. According to different treatments, the 30 rats with DED were divided into three groups: dry eye group with no treatment (DED group, $n=10$); phosphate buffer saline treated group (PBS group, $n=10$); diquafosol treated group (DQS group, $n=10$). The rats in DED group were not treated, both eyes of rats in PBS group were treated with 20 μL PBS solution and both eyes of rats in DQS group were treated with 20 μL diquafosol sodium eye drops (Santen, Osaka, Japan), both 6 times a day for 10d. It is necessary to be mentioned that the 3 animal groups were not installed with PI during the 10 day-long treatment period after the first 14d of PI instillations. We measured clinical parameters on days 3, 7, 10 after treatment, then overdose anesthetic was injected to all rats to collect their eye balls and adnexa for further histological analyses.

Ocular Surface Comprehensive Analyzer Ocular surface comprehensive analyzer (Kanghua, Chongqing, China) was used to detect the tear film stability and lipid film on rats by observing the flowing lipids on the eye surface and Placido ring. We recorded four to five consecutive blink cycles in all. The placido ring collected by camera was a complete concentric ring when tear film was intact, whereas the placido ring was irregular or distorted^[13]. Because ocular surface comprehensive analyzer examination is non-invasive, it was conducted firstly.

Measurement of Tear Production Phenol red cotton threads (Jingming, Tianjin, China) were used to evaluate tear production on days 3, 7, 10 after treatment. After isoflurane anesthesia, phenol cotton thread was immediately inserted under the lower conjunctival fornix of rats for 20s^[14]. The length of the wet red thread which observed with microscope in millimeters was measured to assess amount of tear production. The procedure was repeated at least three times, and the average values were calculated.

Corneal Fluorescein Staining and TBUT Slit lamp microscope with cobalt blue filter (Shangbang, Chongqing, China) was conducted to observe ocular surface. After dropping 2 μL of 1% fluorescein sodium into the lower conjunctival sac, the time(s) from the eyelid opening to the first dry spot turning up was recorded three times in succession. The average value was taken and recorded^[15]. The cornea was separated into four quadrants: supranasal, infranasal, supratemporal and infratemporal. Each quadrant was scored separately. The fluorescein staining score was calculated as below: 0, non; 1, sparse staining less than 30 spots; 2, more than 30 staining spots, but not diffuse; 3, diffuse staining, but no plaque was formed; 4, positive plaques appear, such as ulcers^[16].

In Vivo Confocal Microscopy Examination After rats anesthetized, the examination of cornea was conducted by Heidelberg Retina Tomograph II (HRT). The target area and

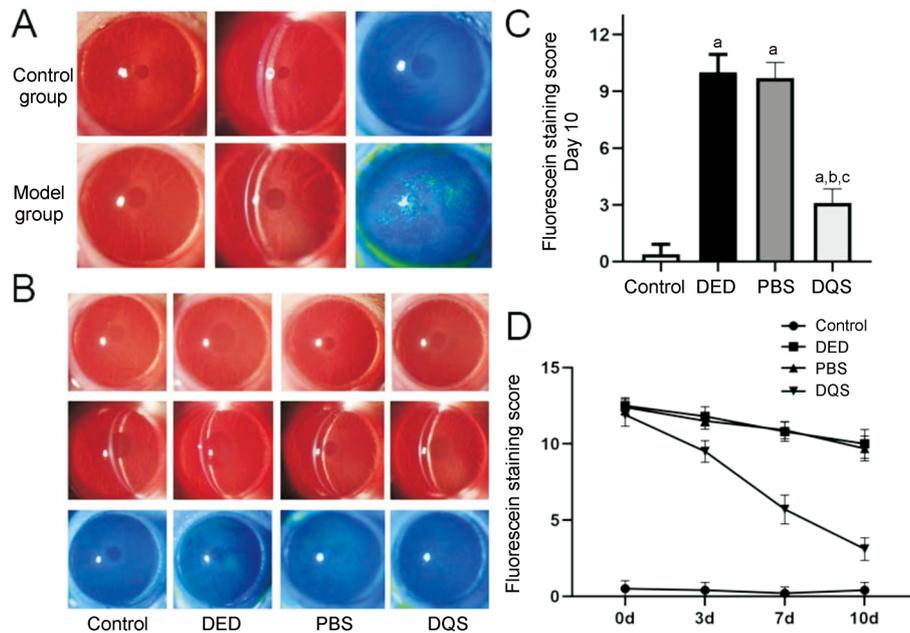


Figure 1 The anterior segment photos and FL staining in each group A: Representative anterior segment photos and FL score in model group for application of PI for 14d, compared with control group; B: Representative anterior segment photos and FL score in control group, DED group, PBS group and DQS group on day 10 of treatment; C: Corneal FL staining score on day 10 of treatment; D: FL staining at different time points of treatment in each group. Each value represents the mean±SD, $n=10$. ^a $P<0.001$ vs control group, ^b $P<0.001$ vs DED group, ^c $P<0.001$ vs PBS group. DED: Dry eye group with no treatment; PBS: Phosphate buffer saline treated group; DQS: Diquafosol treated group; FL: Fluorescein.

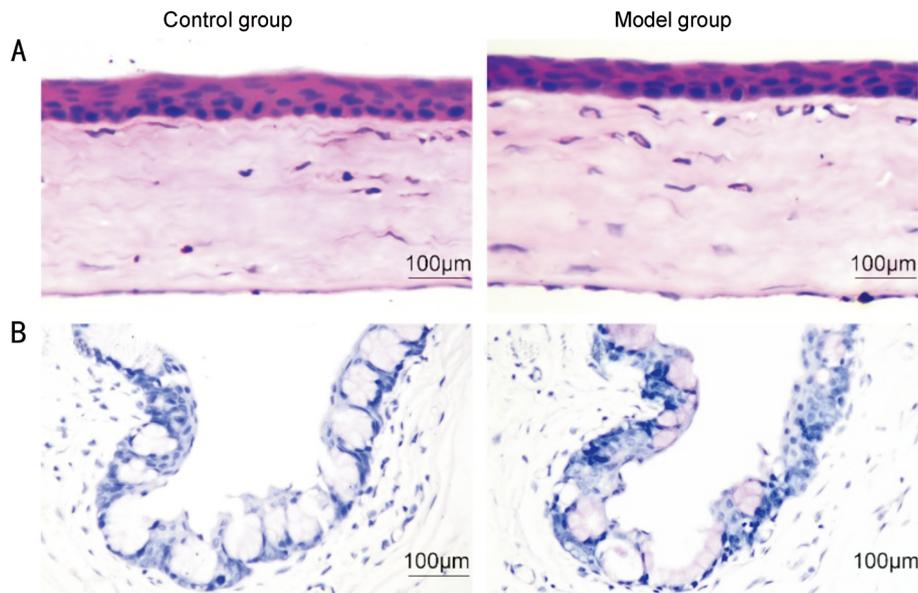


Figure 2 The histologic changes of each group A: The representative images of HE staining of cornea of control group and model group; B: The representative images of PAS staining of conjunctiva of control group and model group. Scale bar: 100 μm . HE: Hematoxylin-eosin; PAS: Periodic acid Schiff.

depth were adjusted manually and images ($400\times 400\ \mu\text{m}^2$ in size) were captured. Images of each layer (epithelium, stroma, endothelium, and nerve fibers) were recorded for each eye. The inflammatory cells were counted on the stromal images among the four groups^[17].

Histology Eyeballs and adnexa were fixed in 4% paraformaldehyde overnight, dehydrated with gradient ethanol successively, embedded with paraffin, then cut to 5- μm

thick sections with microtome (Leica, Nussloch, Germany). HE staining were conducted to observe the corneal and conjunctival epithelium. PAS staining was performed to evaluate density of conjunctival goblet cells^[18-19]. Alcian blue staining were executed to observed the number of mucins on conjunctival sac^[20]. Three sections of 300 μm intervals were selected to stain. All stained sections were recorded with HD digital camera (Nikon, Tokyo, Japan).

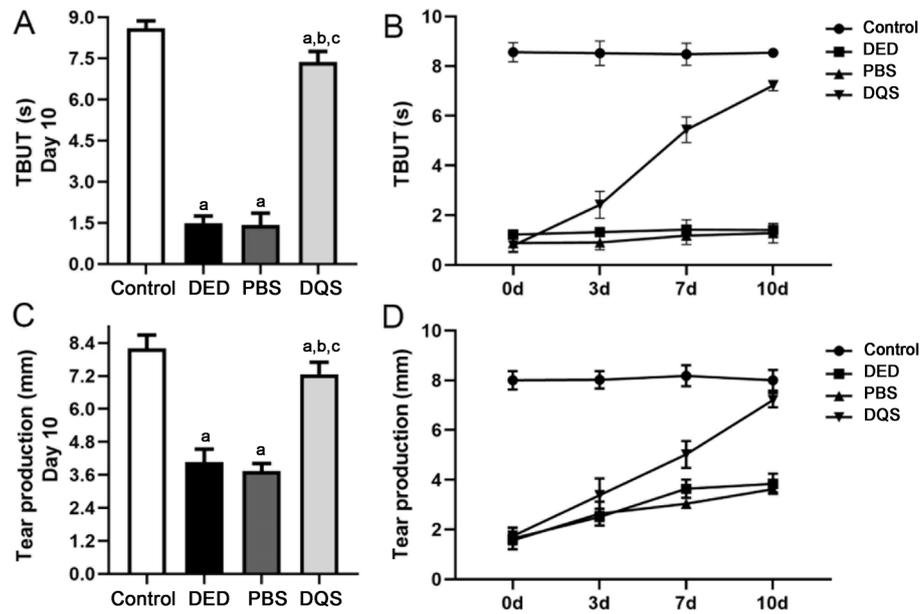


Figure 3 The changes of TBUT and tear production A: TBUT on day 10 of treatment; B: TBUT at different time points of treatment in each group; C: Tear production on day 10 of treatment; D: Tear production at different time points of treatment in each group. Each value represents the mean±SD, n=10. ^aP<0.001 vs control group, ^bP<0.001 vs DED group, ^cP<0.001 vs PBS group. DED: Dry eye group with no treatment; PBS: Phosphate buffer saline treated group; DQS: Diquafosol treated group; TBUT: Tear break-up time.

Ultrastructural Analysis The cornea tissues were fixed in 2.5% glutaraldehyde for 2h and in 10 g/L osmium acid for 2h, dehydrated with gradient ethanol, immersed with epoxy resin, then stained with lead citrate and uranium acetate^[21]. The ultrastructural changes of corneal epithelium were evaluated with transmission electron microscope (TEM). Images were recorded with a camera (Hitachi, Tokyo, Japan).

Immunofluorescence Staining The 5-µm thick paraffinic sections of eyeballs and adnexa were prepared for immunofluorescence staining. The sections were incubated with 0.3% Triton X-100 for 20min, incubated with 1% bovine serum albumin (BSA) for 1h, then incubated with primary antibody mucin 1 (MUC1; 1:250, Santa, Dallas, USA) or polymorphonuclear (PMN; 1:1000, Fitzgerald, USA) overnight. Then sections were incubated with a secondary antibody (1:500; abbkine, Wuhan, China) for 1h. At last sections were counterstained with DAPI (Solarbio, Beijing, China). Images were captured with a fluorescence microscope (Leica, Wetzlar, Germany)^[22].

Examination of Cell Apoptosis with TUNEL Staining For evaluating apoptosis in corneal epithelium, TUNEL assay was performed in 5 µm-thick paraffinic sections of cornea using the *in situ* cell death detection kit fluorescein system (Promega, Wisconsin, USA). Sections were counterstained with DAPI^[23]. Images were obtained using a fluorescence microscope (Leica, Wetzlar, Germany).

Statistical Analysis Statistical analysis was performed using GraphPad Prism version 8.0 (GraphPad Prism, San Diego, CA, USA) and all data were expressed as mean±standard deviation

(SD). Difference between groups were analyzed by one-way ANOVA, and P<0.05 was defined as statistically significant.

RESULTS

Tear Production In comparison to control group, as it turned out, tear production was significantly decreased after application of PI for 14d (7.96±0.422 vs 1.47±0.374 mm; P<0.01). Topical application of diquafosol for 10d significantly increased tear production in rats compared with DED and PBS groups (7.26±0.440 vs 4.07±0.474 mm; 7.26±0.440 vs 3.74±0.280 mm; P<0.01). Besides, there was no significant difference between DED group and PBS group (4.07±0.474 vs 3.74±0.280 mm; P=0.33; Figure 3C, 3D), n=10 for each group.

Corneal Fluorescein Staining and TBUT Compared with control group (8.15±0.564s), TBUT was significantly reduced after application of PI for 14d (0.87±0.307s, P<0.01). After 10d of treatment, DQS group (7.37±0.383s) demonstrated a significant increase in tear film TBUT compared with DED group (1.49±0.260s) and PBS groups (1.42±0.437s, all P<0.01), whereas the PBS group showed no significant improvement compared with the DED group (P=0.07; Figure 3A, 3B).

On day 10, the corneal fluorescein staining scores in the DED, PBS, and DQS groups were 10.0±0.943, 9.7±0.823, and 3.1±0.738, respectively. Treatment with topical diquafosol resulted in a significant decrease in corneal staining scores compared with DED and PBS groups (all P<0.01), indicating a reduction of epithelial injury and restoration of epithelial barrier function (Figure 1B-1D).

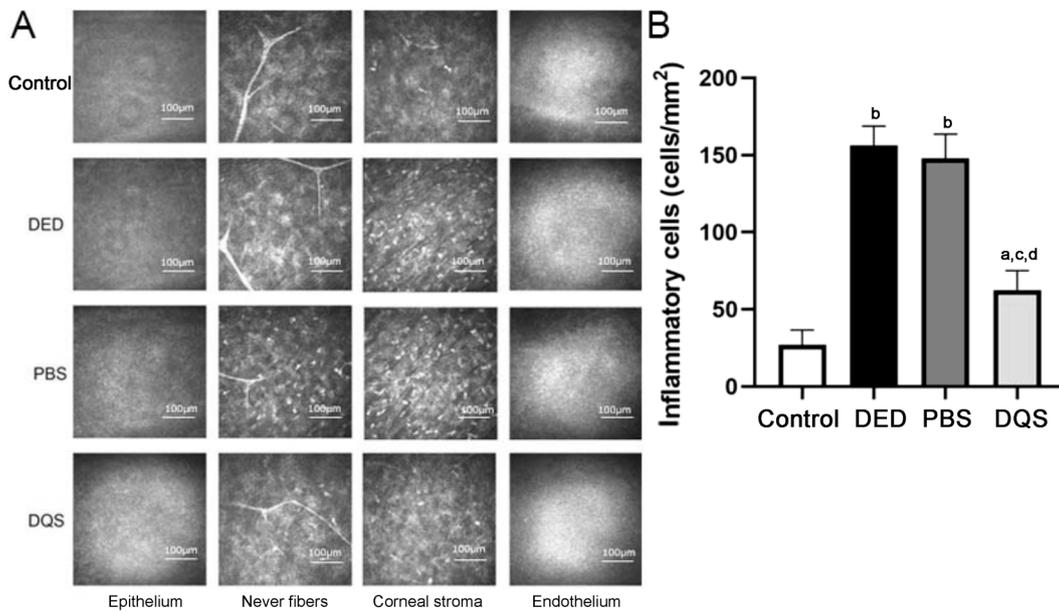


Figure 4 Examination of corneal confocal microscopy A: Representative images of corneal epithelial cells, subepithelial nerve fibers, corneal stroma, and corneal endothelial cells in each group for day 10 of treatment. Scale bar: 100 μ m. B: The number of corneal inflammatory cells of each group. Each value represents the mean \pm SD, $n=3$. ^a $P<0.05$ vs control group, ^b $P<0.001$ vs control group, ^c $P<0.001$ vs DED group, ^d $P<0.001$ vs PBS group. DED: Dry eye group with no treatment; PBS: Phosphate buffer saline treated group; DQS: Diquafosol treated group.

Confocal Microscopy Confocal microscope was used to observe the layers of cornea. In control group, the arrangement of corneal epithelial cells was tight, the neural stems were clear and straight, and endothelial cells were closely arranged. After application of PI, there were bending of neural stem, branches of rats and inflammatory cells infiltration (bright cells) in corneal stroma, in DED (156.3 \pm 12.50) and PBS (147.9 \pm 15.73) groups, whereas the inflammatory cells in DQS group (62.5 \pm 12.50) decreased significantly ($P<0.01$). The result showed that PI could cause inflammatory reaction and destroy corneal nerve fibers, whereas diquafosol could significantly inhibit inflammation, but short-term use of diquafosol had no effect on repairing corneal nerve (Figure 4).

Tear Film Stability Placido ring and lipid of rat ocular surface were observed by ocular surface comprehensive analyzer. Placido ring represents tear film stability. Placido rings were smooth and complete in control and DQS groups, rough and incomplete in PBS and DED groups (Figure 5B), lipid flowing of ocular surface was observed in DQS and control groups, but not in PBS and DED groups. The results showed that PI could destroy tear film stability and inhibit ocular surface lipids secretion, whereas diquafosol could repair tear film stability and increase the secretion of lipid (Figure 5A).

Histopathologic Findings In control group, corneal epithelium was intact and arranged orderly, with 4–6 layers, and endothelial cells arranged in a single layer. Corneal epithelial layers increased in DED and PBS groups, inflammatory cells infiltration and edema were seen in subepithelial stroma. Corneal layers in DQS group were

roughly normal, there was no obvious inflammatory cells infiltration in stroma, and corneal edema was slight. It showed that PI could cause corneal epithelial injury and inflammation, whereas diquafosol could inhibit inflammation and promote corneal epithelial repair (Figure 6A).

Number of Conjunctival Goblet Cell After PAS staining, the conjunctiva and goblet cells of rats in each group were observed by microscope, and the goblet cells were counted by Image J software. In control group, the conjunctival epithelial cells of rats arranged in order, whereas those in DED and PBS groups were irregular, disorganized, and the number of epithelium layers increased. The conjunctival epithelial cells of DQS group arranged orderly, and the number of layers was approximately normal (Figure 6B). In comparison to control group (11.74 \pm 1.346 cells/0.1 mm²), the density of conjunctival goblet cells in DED group (5.21 \pm 0.813 cells/0.1 mm²) and PBS group (5.36 \pm 0.615 cells/0.1 mm²) decreased ($P<0.01$). Compared with DED and PBS groups, the density of goblet cells in DQS group was significantly increased (8.45 \pm 0.718 cells/0.1 mm², $P<0.01$). It showed that PI could cause goblet cells loss and epithelium destruction. Diquafosol could promote the regeneration of goblet cells and repair conjunctival epithelial cells (Figure 6D).

Mucin Secretion in the Conjunctiva After alcian blue staining, the conjunctival mucins of rats in each group were observed by optical microscope, and the mucins were counted by Image J software. Compared with control group (13.45 \pm 1.18 cells/0.1 mm²), the number of mucins in DED group (7.23 \pm 0.614 cells/0.1 mm²) decreased

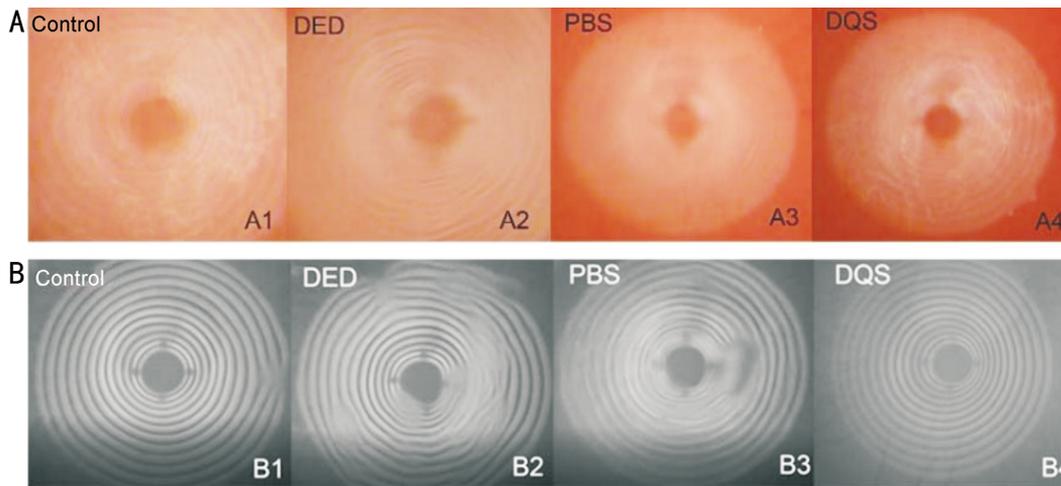


Figure 5 Examination of ocular surface comprehensive analyzer A: Lipid flowing on the ocular surface of each group; B: Placido ring of each group. DED: Dry eye group with no treatment; PBS: Phosphate buffer saline treated group; DQS: Diquafosol treated group.

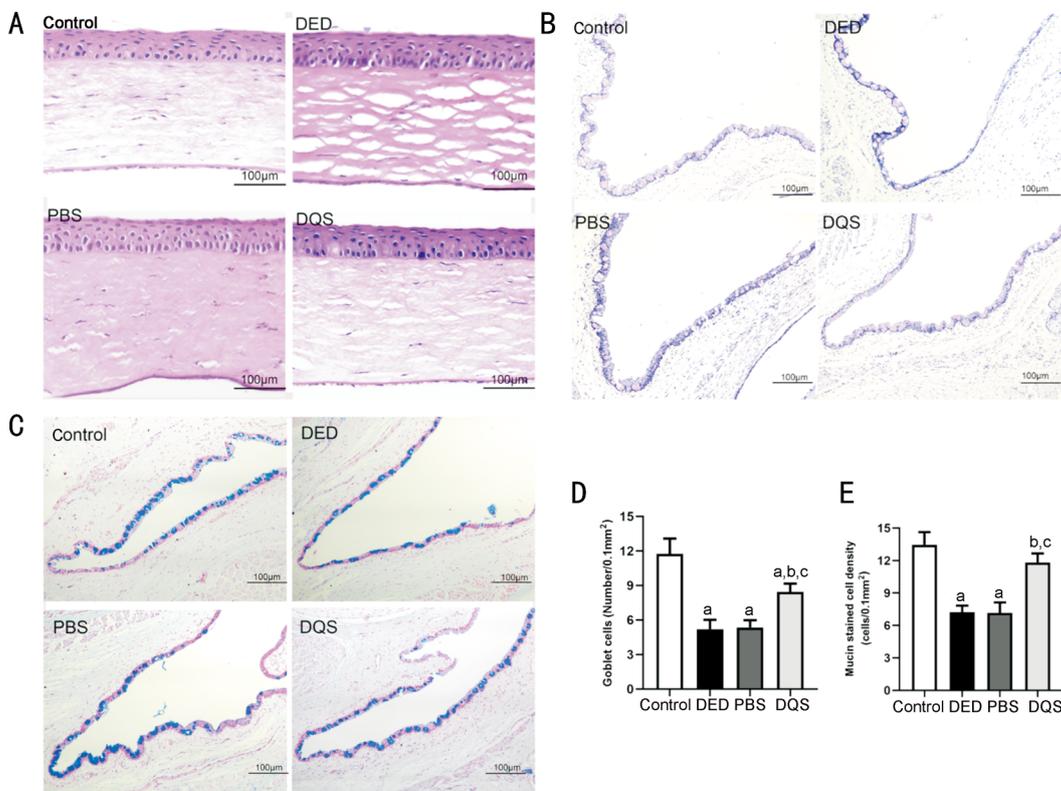


Figure 6 The histologic changes of each group A: The representative images of HE staining of cornea of each group; B: The representative images of PAS staining of conjunctiva of each group; C: The representative images of alcian blue staining of conjunctiva of each group; D: The number of goblet cells of each group; E: The number of mucins of each group. Each value represents the mean±SD, n=5. ^aP<0.001 vs control group, ^bP<0.001 vs DED group, ^cP<0.001 vs PBS group. Scale bar: 100 μm. DED: Dry eye group with no treatment; PBS: Phosphate buffer saline treated group; DQS: Diquafosol treated group; HE: Hematoxylin-eosin; PAS: Periodic acid Schiff.

significantly ($P<0.01$). Compared with DED and PBS groups (7.17 ± 0.968 cells/ 0.1 mm^2), the number of mucins in DQS group (11.83 ± 0.828 cells/ 0.1 mm^2) increased significantly ($P<0.01$). The results showed that PI could inhibit secretion of conjunctival mucin and diquafosol could significantly promote mucin secretion and maintain tear film stability (Figure 6C, 6E). After MUC1 immunofluorescence staining of the ocular sections of rats in each group, the positive expression in cornea

and conjunctiva was observed under fluorescence microscope. Positive expression in cornea and conjunctiva could be observed in control group, and only a little positive expression could be observed in DED and PBS groups. Compared with DED and PBS group, the positive expression of DQS group increased. The results indicated that PI could inhibit MUC1 secretion in cornea and conjunctiva, while diquafosol could promote MUC1 secretion (Figure 7).

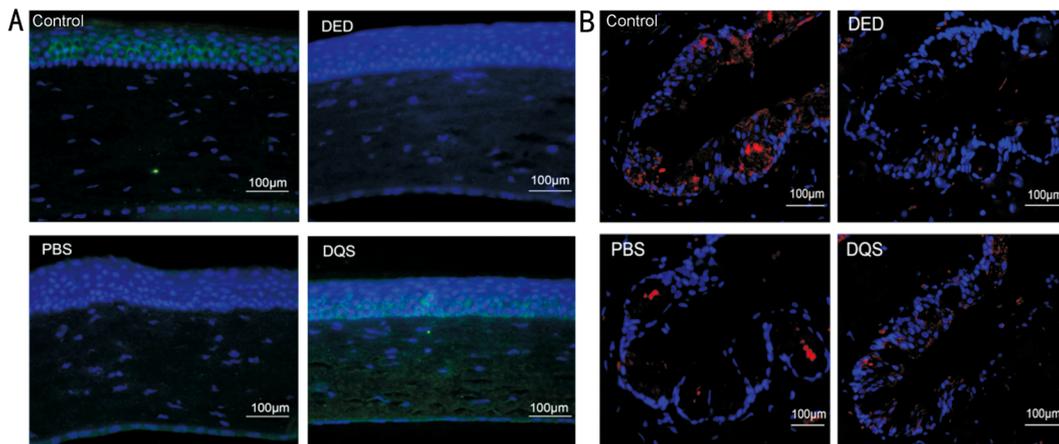


Figure 7 The MUC1 immunofluorescence staining of cornea and conjunctiva of each group A: The MUC1 immunofluorescence staining of cornea of each group; B: The MUC1 immunofluorescence staining of conjunctiva of each group. Scale bar: 100 µm. DED: Dry eye group with no treatment; PBS: Phosphate buffer saline treated group; DQS: Diquafosol treated group; HE: Hematoxylin-eosin; PAS: Periodic acid Schiff; MUC1: Mucin 1.

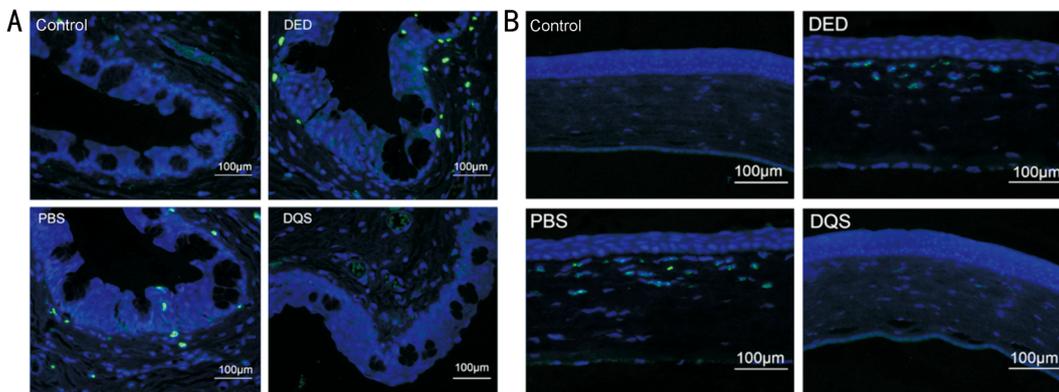


Figure 8 The PMN immunofluorescence staining of cornea and conjunctiva of each group A: The PMN immunofluorescence staining of cornea of each group; B: The PMN immunofluorescence staining of conjunctiva of each group. Scale bar: 100 µm. DED: Dry eye group with no treatment; PBS: Phosphate buffer saline treated group; DQS: Diquafosol treated group; PMN: Polymorphonuclear.

Inflammatory Cells Infiltration The positive expression of PMN (polymorphonuclear) in cornea and conjunctiva of rats in each group was observed under fluorescence microscope. No positive expression was found in control group, a large amount of expression was found in DED and PBS groups and positive expression decreased significantly in DQS group. The results indicated that PI could induce inflammation in the cornea and conjunctiva, and diquafosol could significantly inhibit inflammatory cells infiltration (Figure 8).

Apoptosis of Corneal Epithelium Cells After TUNEL staining, positive apoptotic cells were evaluated using fluorescence microscope. A few of apoptotic cells were observed in corneal epithelium of control and DQS groups, plenty of apoptotic cells were observed in the cornea of DED and PBS groups. The results showed that PI could induce corneal cell apoptosis, and diquafosol could effectively inhibit corneal cell apoptosis (Figure 9).

Effect of DQS in Changing Ultra-Structure in Cornea In control group, microvillus extended in finger shape and arranged neatly on corneal epithelium, and intercellular

connection was tight. In DED and PBS groups, only a few microvilli on the corneal epithelium, the desmosome structure was destroyed, and the intercellular connection was interrupted. The number of microvillus and desmosomes increased and cell junction partially recovered in DQS group. The results showed that PI could destroy the microvilli and desmosome structure, and diquafosol could repair the ultrastructure of cornea, promote formation of microvillus and desmosomes, and restore corneal intercellular connection (Figure 10).

DISCUSSION

In ophthalmic surgery, the ultimate purpose of using disinfectants is to prevent infectious endophthalmitis and PI is the most commonly used disinfectant^[24]. However, there is no standard on the optimal time, concentration and dose of PI solution for ophthalmic surgery disinfection. PI is used in ophthalmic surgery at a variety of concentrations (0.01%–10%), duration (30s–3min) and frequency (once or twice)^[25–26]. At present, exposure to 0.5% PI solution for 5min is considered to be the choice of eye disinfection before corneal transplantation^[27]. The ocular surface damage caused by PI

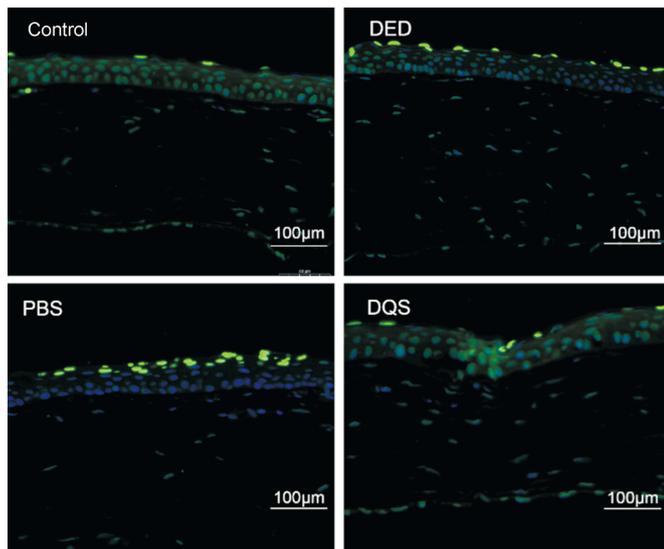


Figure 9 The TUNEL staining of cornea of each group Scale bra: 100 µm. DED: Dry eye group with no treatment; PBS: Phosphate buffer saline treated group; DQS: Diquafosol treated group.

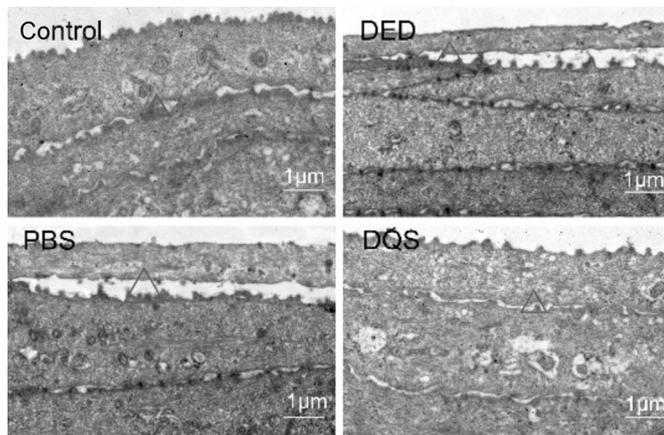


Figure 10 The transmission electron microscope of cornea of each group Scale bar: 1 µm. DED: Dry eye group with no treatment; PBS: Phosphate buffer saline treated group; DQS: Diquafosol treated group.

infusion before cataract surgery, including goblet cell loss and corneal epithelial damage, is comparable to that of dry eye^[28-29]. Therefore, we hypothesized that povidone iodine solution may be the pathogenic factor of dry eye in patients undergoing ophthalmic surgery.

We successfully induced the rat dry eye model in this study by dropping 10 g/L PI solution twice a day for 14d. The DED group had shorter tear film TBUT, higher fluorescein staining score and less tear production. The rougher Placido ring indicated the PI could disturb tear film stability. Based on pathological findings, we demonstrated that PI could damage corneal and conjunctival epithelium, induce inflammation reaction in cornea and conjunctiva, inhibit mucin secretion, destroy corneal ultrastructure and increase curvature and branching of corneal nerve fibers.

All of these alterations showed that changes of rat's ocular surface were similar to symptoms of human dry eye syndrome.

The inflammation, conjunctival mucin loss and epithelial cell apoptosis caused by PI solution are very important in the pathogenesis of DED. Our dry eye model provided a wealth of information for investigating inflammatory, apoptosis, and mucin reduction mechanisms of dry eye.

The major function of goblet cells is to generate and secrete mucins. Mucins are linked to the microvillus of the epithelial cells^[30], playing a critical function in maintaining hydrophilicity of tear film. It maintains the integrity of the tear film and decreases the surface tension, allowing a well-distributed distribution of the aqueous layer over the ocular surface^[31]. The loss of mucin could accelerate tear evaporation, contribute to tear hyperosmolarity, eventually lead to inflammation. Thus, microvilli degradation and mucin loss generated by PI could interfere the formation of mucosal layer and increase tear evaporation.

By acting on P2Y2 receptors on ocular surface and increasing intracellular Ca²⁺ concentrations, diquafosol promotes tear and mucin production^[31]. It also improves fluid transport, eventually improve quantity and quality of tear film.

In DQS group, TBUT and tear production increased and corneal fluorescein staining score decreased. We observed lipid flowing on ocular surface, indicating that diquafosol could promote lipid secretion and stabilize tear film. Meanwhile, corneal and conjunctival inflammation cells infiltration significantly decreased, indicating that diquafosol could inhibit stromal inflammatory infiltration. Last but not least, diquafosol could inhibit corneal epithelium apoptosis and goblet cells loss, promote mucin secretion, repair damaged corneal epithelium and maintain tear film stability.

In conclusion, our study demonstrated PI could induce clinical symptoms and histopathological changes of dry eye. In our previous study, 10% PI solution instillation of Sprague Dawley rats once could cause corneal edema and exfoliation of the corneal epithelium right away. The toxicity of PI in cornea should be attach importance by ophthalmologists. The results showed that diquafosol could alleviate symptoms in the PI-induced rat dry eye model. Furthermore, diquafosol has the potential to reduce inflammation, inhibit corneal epithelial apoptosis, increase goblet cell density, and restore mucin secretion.

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Conflicts of Interest: Zhang C, None; Wang H, None; Chen DY, None; Zhao K, None; Wang W, None; Li MX, None.

REFERENCES

- 1 Walter K. What is dry eye disease? *JAMA* 2022;328(1):84.
- 2 Hakim FE, Farooq AV. Dry eye disease: an update in 2022. *JAMA* 2022;327(5):478-479.
- 3 Kojima T, Dogru M, Kawashima M, Nakamura S, Tsubota K. Advances in the diagnosis and treatment of dry eye. *Prog Retin Eye Res* 2020:100842.

- 4 Baudouin C, Rolando M, Benitez Del *et al.* Reconsidering the central role of mucins in dry eye and ocular surface diseases. *Prog Retin Eye Res* 2019;71:68-87.
- 5 Baudouin C, Irkeç M, Messmer EM, *et al.* Clinical impact of inflammation in dry eye disease: proceedings of the ODISSEY group meeting. *Acta Ophthalmol* 2018;96(2):111-119.
- 6 González Martín-Moro J, Zarallo-Gallardo J, Guzmán-Almagro E, Antón Rodríguez C. A systematic review and meta-analysis on the efficacy of topical povidone iodine in adenoviral conjunctivitis. *Cont Lens Anterior Eye* 2023;46(5):101873.
- 7 Kim S, Ahn Y, Lee Y, Kim H. Toxicity of Povidone-iodine to the ocular surface of rabbits. *BMC Ophthalmol* 2020;20(1):359.
- 8 Nakamura M, Imanaka T, Sakamoto A. Diquafosol ophthalmic solution for dry eye treatment. *Adv Ther* 2012;29(7):579-589.
- 9 Eom Y, Kim HM. Clinical effectiveness of diquafosol ophthalmic solution 3% in Korean patients with dry eye disease: a multicenter prospective observational study. *Int J Ophthalmol* 2021;14(10):1518-1526.
- 10 Kang DH, Lee YW, Hwang KY, *et al.* Changes of tear film lipid layer thickness by 3% diquafosol ophthalmic solutions in patients with dry eye syndrome. *Int J Ophthalmol* 2019;12(10):1555-1560.
- 11 Matsumoto Y, Ohashi Y, Watanabe H, Tsubota K. Efficacy and safety of diquafosol ophthalmic solution in patients with dry eye syndrome: a Japanese phase 2 clinical trial. *Ophthalmology* 2012;119(10):1954-1960.
- 12 Ohashi Y, Munesue M, Shimazaki J, *et al.* Long-term safety and effectiveness of diquafosol for the treatment of dry eye in a real-world setting: a prospective observational study. *Adv Ther* 2020;37(2):707-717.
- 13 Mu N, Wang H, Chen DY, Wang F, Ji L, Zhang C, Li MX, Lu PR. A novel rat model of dry eye induced by aerosol exposure of particulate matter. *Invest Ophthalmol Vis Sci* 2022;63(1):39.
- 14 Liu JY, Chu HS, Wei YH, *et al.* Review, analysis, and education of antiseptic related ocular injury in the surgical settings. *Ocul Surf* 2021;22:60-71.
- 15 Xiao XY, He H, Lin ZR, Luo PP, He H, Zhou T, Zhou YP, Liu ZG. Therapeutic effects of epidermal growth factor on benzalkonium chloride-induced dry eye in a mouse model. *Invest Ophthalmol Vis Sci* 2012;53(1):191.
- 16 Lee H, Shim W, Kim CE, Choi SY, Lee H, Yang J. Therapeutic efficacy of nano complex of poly(ethylene glycol) and catechin for dry eye disease in a mouse model. *Invest Ophthalmol Vis Sci* 2017;58(3):1682.
- 17 Matsumoto Y, Ibrahim OMA. Application of *in vivo* confocal microscopy in dry eye disease. *Invest Ophthalmol Vis Sci* 2018;59(14):DES41-DES47.
- 18 Bhattacharya D, Ning Y, Zhao FK, Stevenson W, Chen RJ, Zhang JS, Wang MW. Tear production after bilateral main lacrimal gland resection in rabbits. *Invest Ophthalmol Vis Sci* 2015;56(13):7774.
- 19 Chen XM, Kuang JB, Yu HY, Wu ZN, Wang SY, Zhou SY. A novel rabbit dry eye model induced by a controlled drying system. *Trans Vis Sci Tech* 2021;10(4):32.
- 20 Kim CE, Kim YJ, Hwang MW, Park YJ, Yang J. Cevimeline-induced anti-inflammatory effect through upregulations of mucins in the ocular surface of a dry eye mouse model. *Biomed Pharmacother* 2021;139:111571.
- 21 Lee H, Jeon S, Kim CE, Park YJ, Yang J. A new ophthalmic pharmaceutical formulation, topical sulglycotide, enhances the ocular mucin secretion in desiccation stress-mediated dry eye disease. *Invest Ophthalmol Vis Sci* 2019;60(4):1076.
- 22 Li CY, Song YY, Luan SH, *et al.* Research on the stability of a rabbit dry eye model induced by topical application of the preservative benzalkonium chloride. *PLoS One* 2012;7(3):e33688.
- 23 Lin ZR, Liu XC, Zhou T, Wang YH, Bai L, He H, Liu ZG. A mouse dry eye model induced by topical administration of benzalkonium chloride. *Mol Vis* 2011;17:257-264.
- 24 Friedman DA, Mason JO, Emond T, McGwin G. Povidone-iodine contact time and lid speculum use during intravitreal injection. *Retina* 2013;33(5):975-981.
- 25 Grzybowski A, Kanclerz P, Myers WG. The use of povidone-iodine in ophthalmology. *Curr Opin Ophthalmol* 2018;29(1):19-32.
- 26 Kramer A, Below H, Behrens-Baumann W, Müller G, Rudolph P, Reimer K. New aspects of the tolerance of the antiseptic povidone-iodine in different *ex vivo* models. *Dermatology* 2002;204(Suppl. 1):86-91.
- 27 Fahmy AM, Hardten DR. Treating ocular surface disease: new agents in development. *Clin Ophthalmol* 2011;5:465-472.
- 28 Chen WT, Chen YY, Hung MC. Dry eye following femtosecond laser-assisted cataract surgery: a meta-analysis. *J Clin Med* 2022;11(21):6228.
- 29 Kanclerz P, Grzybowski A. The use of povidone-iodine in ophthalmology and particularly cataract surgery. *Surv Ophthalmol* 2019;64(3):441-442.
- 30 Li XZ, Kang B, Woo IH, Eom Y, Lee HK, Kim HM, Song JS. Effects of topical mucolytic agents on the tears and ocular surface: a plausible animal model of mucin-deficient dry eye. *Invest Ophthalmol Vis Sci* 2018;59(7):3104-3114.
- 31 Wang WY, Sun XN, Zhao JY, *et al.* 3% diquafosol sodium eye drops in Chinese patients with dry eye: a phase IV study. *Front Med (Lausanne)* 2023;10:1089613.