Toxicity of Povidone-Iodine to the Ocular Surface of Rabbits

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Abstract

Background We evaluated the toxicity of 5% (w/v) povidone-iodine (PI) applied to the ocular surface of rabbits. Methods Twenty three white rabbits were divided into four groups; these were a control group and three study groups in which the ocular surface was exposed to PI for different times. In control group, phosphate-buffered saline (PBS) was applied once for 10 minutes. In study groups, 5% (w/v) PI was topically applied once for 1 minute, 3 minutes, and 10 minutes, and then the animals were observed for 7 days. The Schirmer test, Rose Bengal staining, corneal fluorescein staining and conjunctival impression cytology were performed on day 0, 3, and 7. After 7 days, the rabbits were sacrificed and conjunctiva and cornea were collected and evaluated by light and electron microscope. Immunofluorescence staining was also performed to detect mucin 5 subtype AC (MUC5AC). Results The decrease in goblet cell density, reductions in MUC5AC level and histopathological and ultrastructural changes of conjunctiva and cornea were more prominent in the 5% (w/v) PI groups than the control group ($p < 0.05$). Moreover, these changes were more prominent when PI was applied for 3 and 10 minutes rather than 1 minute (both p values < 0.05). Conclusions 5% (w/v) povidone-iodine caused damages to the ocular surface in a time-dependent manner. Therefore, we should be aware of that excessive PI exposure during ophthalmic procedures could be a pathogenic factor of dry eye syndrome after surgery.

Background

Many factors may affect the ocular surface environment after cataract surgery and many patients have complained of dry eye and symptoms of irritations postoperatively. Khanal et al. reported that disturbances in corneal sensitivity and tear physiology were observed immediately after phacoemulsification.[1] Li et al. proposed that misuse of eye drops could be the major factor causing dry eye symptoms after such surgery.[2] We have already shown that preservatives such as benzalkonium chloride in eye drops can damage the ocular surface in a dose-dependent manner.[3] Corneal denervation caused by surgical incision may also be a risk factor for development of dry eye associated with cataract surgery.[4] In our previous study, the duration of cataract surgical time was highly correlated with development of ocular surface damage such as goblet cell loss and tear film instability.[5] Also, light from the operating microscope was shown to exert phototoxic effects on the ocular surface and the tear film in vivo. Therefore, excessive light exposure during ophthalmic procedures may be one pathogenic factor causing dry eye syndrome after cataract surgery.[6] Of the many factors causing ocular surface damage during such surgery, we proposed that soaking in 5% (w/v) povidone-iodine (PI) may be one of the major causes a major cause of such damage. Recently, topical polyvinylpyrrolidone-iodine (PI) has become widely used as a disinfectant in most fields of ophthalmic surgery, for preoperative prevention of endophthalmitis. The latest guidelines of the European Society of Cataract and Refractive Surgeons (ESCRS) recommend preoperative use of 5% (w/v) PI for a minimum of 3 minutes, or longer application of a sponge pad soaked in the material, to ensure corneal and conjunctival antisepsis (ESCRS version 2; Dublin, 2007).[7] Previous studies have shown that application of 5% (w/v) PI to the cornea and conjunctiva effectively decreased the bacterial load of the ocular surface and the adnexae, and thus

theoretically reduced the risk of postoperative endophthalmitis.[7–10] However, the product has an acidic pH (approximately 3.5), and preoperative PI has been shown to exert cytotoxic effects on the ocular surface epithelial cell.[11, 12] Thus, in the present study, we evaluated the effects of 5% (w/v) PI on the corneal and conjunctival surfaces of rabbit eyes, and ocular surface changes were compared based on PI exposure time.

Methods

All experimental procedures were performed according to the guidelines of the Association for Research in Vision and Ophthalmology (ARVO) as set out in the “Statement for the Use of Animals in Ophthalmic and Visual Research” and the ARRIVE Guidelines for reporting animal research, and approved by the institutional animal care and use committee of Catholic university of Korea. Twenty three New Zealand white male rabbits (DooYeol Biotech, Seoul, Korea) weighing between 2 and 2.5 kg were used in the study. The rabbits were housed in the individual mesh cages and given free access to standard laboratory food and water. They were housed in an air-conditioned room under a 12 h-light/12 h-dark cycle. No rabbit had either a corneal or conjunctival disorder. A commercially available 10% (w/v) PI solution was diluted with distilled water to 5% (w/v). Rabbits were randomly divided into four groups, with topical administration of phosphate-buffered saline (PBS) for 10 minutes in the control group (ten eyes), and topical administration of 5% (w/v) PI for 10 minutes, 3 minutes, and 1 minute in groups 1, 2, and 3, respectively (twelve eyes each). A mixture of tiletamine and zolazepam (Zoletil®, Virbac, Carros, France) at 0.2 mg/kg was injected intramuscularly to immobilize the rabbits prior to performing the Schirmer test, Rose Bengal staining, corneal fluorescein staining and conjunctival impression cytology (CIC), and proxymetacaine hydrochloride 0.5% (w/v) eye drops (Alcaine®, Alcon Laboratories Inc, Fort Worth, TX, USA) were applied topically.

Fifteen among the twenty three rabbits (6 control eyes and 8 eyes each from three PI groups) underwent Schirmer test, fluorescein staining, Rose Bengal staining, conjunctival impression cytology(CIC) and conjunctival histological analysis by light and transmission electron microscopy, and conjunctival immunofluorescence staining for MUC5AC was performed. The Schirmer test was performed at baseline (day 0) and after PI instillation on day 3 and 7. Fluorescein staining and Rose Bengal staining were performed on day 0 (immediately after PI treatment), 3, and 7. Although Rose Bengal stain pre-PI treatment data are required for an accurate comparison, it was not applied because all cornea and conjunctiva were considered healthy by slit lamp microscopy before instillation of povidone, and Rose Bengal would have caused ocular surface damage. Thus, the analysis was made by comparison with the control group.

In addition, CIC was performed on the upper bulbar conjunctiva after PI treatment on day 0, 3, and 7. After day 7, conjunctival tissues were collected for histological analysis by light and transmission electron microscopy and immunofluorescence staining was performed to detect the mucin 5 of subtype AC (MUC5AC).

Other eight rabbits (4 control eyes and 4 eyes each from three PI groups) underwent corneal fluorescein staining. After day 7, the cornea was carefully excised and corneal damage was examined using electron microscopy.

Before the collection of cornea and conjunctival tissues, all animals were killed by intraperitoneal injection of a lethal overdose of pentobarbital (100 mg/kg body weight).

The Schirmer Test And Rose Bengal Staining

A standard Schirmer test strip (the Color Bar^{TM}; Eagle Vision, Memphis, TN) was placed inside the margin of the inferolateral one-third of the lower eyelid. The amount of moisture in the strip was measured after 5 minutes. The Schirmer test value was determined by measuring the length of the moistened portion of the strip. Rose Bengal staining was performed according to a method that has previously described.[13] Briefly, after administration of 2 µL of 1% (w/v) Rose Bengal (Sigma, St. Louis, MO) into the conjunctival sac, the extent of staining of the ocular surface was microscopically (Opmi6-CFC; Carl Zeiss AG, Jena, Germany) graded using the Van Bijsterveld grading system, which sums the staining grades of the nasal conjunctiva, the cornea, and the lateral conjunctiva; the maximum score is 3 per region and the maximum total score is thus 9.[14]

Corneal Fluorescein Staining

Two microliters of 1% sodium fluorescein were instilled into the lower fornix of the conjunctiva. The rabbit was allowed to blink several times to distribute the fluorescein evenly on the cornea. Two minutes later, corneal fluorescein staining intensity was also examined and graded under a portable slit-lamp microscope (Kowa SL–15; Kowa, Tokyo, Japan). The grading was based upon a scale of 0 to 3 in five areas of the cornea: central, superior quadrant, inferior quadrant, nasal quadrant and temporal quadrant. The maximum possible score was 15.

Conjunctival Impression Cytology

To perform CIC, a 4 3-mm mixed cellulose ester membrane (ADVANTEC; Toyo Roshi Kaisha Ltd., Tokyo, Japan) was placed on the upper bulbar conjunctiva and constant pressure was applied for 5 seconds. Conjunctival impression cytology (CIC) is conducted only in the upper bulbar conjunctiva, as it is the most convenient site to reveal rabbit conjunctiva and collect a sufficient number of cells. This process was performed at the same place on the upper bulbar conjunctiva on all days. The membrane was gently lifted off and fixed in 10% (v/v) formaldehyde. Hematoxylin and the periodic acid–Schiff (PAS) reagents were used to stain all specimens. After staining, the goblet cell density (GCD) was calculated and the morphology of the conjunctival epithelium was graded using the Nelson system with the aid of a Zeiss Axioskop 40 microscope (Carl Zeiss, Oberkochen, Germany) fitted with an 40 objective.[15] Three different sections of each specimen were randomly selected for goblet cell counting, and averages were calculated [cells/high-power (HP) visual field (400)].

Light Microscopy Of Conjunctival Tissue And Electron Microscopy Of Corneal And Conjunctival Tissue

For light microscopic examination, bulbar conjunctival tissues were collected on day 7 and fixed in 10% (v/v) formaldehyde. After dehydration, specimens were embedded in paraffin, cross-sectioned, stained with hematoxylin and the PAS reagents, and evaluated under a Zeiss Axioskop 40 microscope fitted with a 40 objective. For transmission electron microscopic examination, conjunctival specimens were fixed in 2% (v/v) paraformaldehyde/2.5% (v/v) glutaraldehyde in 0.1 M phosphate buffer (pH 7.4), post-fixed in osmium tetroxide (0.1 M in phosphate buffer), embedded in epoxy resin, and cut into ultrathin sections. These sections were double-stained with uranyl acetate and lead citrate. Specimens were viewed and photographed using a transmission electron microscope (TEM 1010; JEOL Ltd., Tokyo, Japan). Harvested corneal tissues were prepared for scanning electron microscopy (SEM). For SEM, fixed corneas were dehydrated in increasing concentrations of acetone. The fixed specimens were critical-point dried, mounted on metal stubs with conductive silver paint, and then sputtered with a 10-nm-thick layer of gold in an ion sputter (JFC–1100; JEOL). The corneas were examined with a Hitachi S–4700 scanning electron microscope (Hitachi High-Technologies, Tokyo, Japan) at an acceleration voltage of 10 kV.

Immunofluorescence Staining For Muc5ac

Immunodetection of MUC5AC was performed by immunofluorescence staining of sections prepared from paraffin blocks of the conjunctiva after experimental animals had been sacrificed on day 7. Corneal specimens served as one set of negative controls. After samples were deparaffinized and rehydrated in xylene (first) and ethanol (next), slides were heated in boiling citrate buffer [10 mM citric acid, 0.05% (v/v) Tween–20 (pH 6.0)]; and next treated (first) with 3% (v/v) H_2O_2, and (second) with peroxidase blocking solution [10% (v/v) FBS in PBS with 20 mM HEPES (4-(2-hydroxyethyl)–1-piperazineethanesulfonic acid)]. After three washes in PBS, the slides were blocked with 1% (v/v) fetal bovine serum for 30 minutes at room temperature, followed by incubation for 12 h at 4°C with a 1:100 dilution of mouse anti-human MUC5AC antibody (45M1, catalog no. ab11335; Abcam, Cambridge, UK). After three further washes in PBS, the slides were incubated with fluorescein isothiocyanate (FITC)-conjugated secondary goat anti-mouse IgG (Abcam) for 45 minutes at room temperature. Next, nuclear counterstaining was performed using a 0.5 μg/mL solution of Hoechst 33342 dye (Invitrogen, Carlsbad, CA). Finally, specimens were observed under a fluorescence microscope.

Statistical Analysis

Statistical analysis was performed using SPSS version 18.0 (SPSS Inc., Chicago, IL). The statistical analysis on differences between individual groups based on time elapsed was conducted with repeated-measures analysis of variance (ANOVA) and Friedman's test. The Rose Bengal test, corneal fluorescein staining score and Nelson scores of the various groups were compared using the Steel–Dwass test. The Tukey–Kramer test was used to compare goblet cell counts among the groups. A *p*-value less than 0.05 was considered to indicate statistical significance.

Results

1. Schirmer test values

The baseline Schirmer test values were 16.17 ± 4.00 in the control group, 15.50 ± 3.90 in the 1-minute PI group, 17.67 ± 6.80 in the 3-minutes PI group, and 19.33 ± 4.60 in the 10-minutes PI group. No significant differences were evident among the three PI groups at baseline (p = 0.882). Also, no significant differences were noted among the four groups on day 3 and 7 (p = 0.652 on day 3; 17.50 ± 3.40 in the control group, 15.72 ± 5.40 in the 1-minute PI group, 16.06 ± 4.60 in the 3-minutes PI group, and 16.00 ± 3.00 in the 10-minutes PI group; p = 0.772 on day 7; 19.50 ± 4.30 in the control group, 16.72 ± 3.70 in the 1-minute PI group, 15.50 ± 2.40 in the 3-minutes PI group, and 13.00 ± 5.40 in the 10-minutes PI group).

2. Rose Bengal staining

The Rose Bengal staining scores differed among the four groups on day 0, 3, and 7 (on day 0; 0.08 ± 0.12 in the control group, 1.56 ± 0.60 in the 1-minute PI group, 3.22 ± 0.46 in the 3-minutes PI group, and 5.56 ± 0.56 in the 10-minutes PI group; on day 3; 0.33 ± 0.33 in the control group, 1.44 ± 0.60 in the 1-minute PI group, 3.00 ± 0.58 in the 3-minutes PI group, and 4.00 ± 0.47 in the 10-minutes PI group; on day 7; 0.00 ± 0.00 in the control group, 0.89 ± 0.26 in the 1-minute PI group, 1.89 ± 0.59 in the 3-minutes PI group, and 3.56 ± 0.78 in the 10-minutes PI group, Figure 1). However, significant differences were observed between the control and 3-minutes PI group, between the 3-minutes and 10-minutes PI groups, and between the 1-minute and 10-minutes PI groups only immediately after instillation (p = 0.042, p = 0.040, p = 0.016, respectively, according to the Steel–Dwass test), and no significant differences were observed between the control and the 1-minute PI group, and between the 1-minute and the 3-minutes PI group (p = 0.155, p = 0.582, respectively, according to the Steel–Dwass test). There were no significant differences between the four groups on day 3 and 7 (p > 0.05 in the Steel–Dwass test). Overall, the area stained with Rose Bengal tended to increase as PI exposure time increased. And the Rose Bengal staining showed a tendency to improve with passage of time in all PI groups. However, only the 10-minutes PI group was statistically significant (p = 0.607 in control group, p = 0.905 in 1-minute PI group, p = 0.513 in 3-minutes PI group, and p = 0.04 in 10-minutes PI group by Friedman's test).

3. Corneal Fluorescein Staining

The corneal fluorescein staining scores were counted of 0.5, 5.5, 5.0 and 8.5 each in the control group, 1-minute, 3-minutes and 10-minutes PI group, respectively on day 0, 0.25, 2.5, 2.75 and 7.5 respectively on day 3, and 0.25, 1.5, 2.5 and 7.25 respectively on day 7 (Figure 2). On day 3, some eyes of the 10-minutes PI group showed large epithelial defect on superficial cornea and no improvement until day 7 (Figure 2d). The corneal fluorescein staining score showed significant improve with passage of time only in the 1-minute and 3-minutes PI groups (p = 0.607 in control group, p = 0.038 in 1-minute PI group, p = 0.022 in 3-minutes PI group, and p = 0.584 in 10-minutes PI group by Friedman's test). The control and three PI

groups showed no significant differences in the corneal fluorescein staining score at each time point (p > 0.05 by the Steel Dwass test). However, there were obvious corneal epithelial damages on some eyes of the 10-minutes PI group compared with the other groups.

4. Conjunctival impression cytology

1) Goblet cell density (GCD)

The baseline GCD in the conjunctiva, before any instillation, was 92.3 ± 15 in the control group, 88.6 ± 11.2 in the 1-minute PI group, 92.4 ± 13.9 in the 3-minutes PI group, and 93.7 ± 8.3 in the 10-minutes PI group. And when the GCD of the four groups were compared immediately after the PI instillation (on day 0), there were no significant differences between the four groups (p values of Tukey-Kramer test were 0.971, 0.996, 0.998, 0.976, 0.931 and 0.663 in the control vs 1-minute PI group, control vs 3-minutes PI group, control vs 10-minutes PI group, 1-minute vs 3-minutes PI group, 1-minute vs 10-minutes PI group, 3-minutes vs 10-minutes PI group, respectively).

However, there were apparent significant differences on day 3 and 7 after PI instillation between the control and 3-minutes PI group, the control and 10-minutes PI group, the 1-minute and 3-minutes PI group, the 1-minute and 10-minutes PI group, and the 3-minutes and 10-minutes PI group (all $p < 0.001$ in the Tukey–Kramer test), whereas the control and 1-minute PI group were not different significantly ($p = 0.560$ on day 3, $p = 0.868$ on day 7 in the Tukey–Kramer test). On day 3, the GCD was 87.7 ± 7.4 in the control group, 80.9 ± 7.4 in the 1-minute PI group, 30.5 ± 11.2 in the 3-minutes PI group, and 10.3 ± 6.2 in the 10-minutes PI group. On day 7, the GCD was 76.9 ± 8.2 in the control group, 80.1 ± 10.8 in the 1-minute PI group, 50.2 ± 12.1 in the 3-minutes PI group, and 8.6 ± 8.8 in the 10-minutes PI group (Figure 3).

2) Severity of squamous metaplasia noted by conjunctival impression cytology

When the severity of squamous metaplasia was compared using the Nelson grading system, the Nelson score tended to increase with PI exposure time on day 3 and 7. The Nelson score of the conjunctiva was 0.00 ± 0.00 in all group on day 0. On day 3, the Nelson score of the conjunctiva was 0.20 ± 0.30 in the control group, 0.87 ± 0.20 in the 1-minute PI group, 1.70 ± 0.30 in the 3-minutes PI group, and 2.20 ± 0.40 in the 10-minutes PI group. On day 7, the Nelson score of the conjunctiva was 0.33 ± 0.30 in the control group, 0.70 ± 0.40 in the 1-minute PI group, 1.80 ± 0.40 in the 3-minutes PI group, and 2.40 ± 0.45 in the 10-minutes PI group. The Nelson score was significantly different between the 1-minute and 3-minutes PI group and between the 1-minute and 10-minutes PI groups on day 7 ($p = 0.013$, $p = 0.0031$ by Steel–Dwass test), whereas no significant difference was observed between the control and 1-minute PI group

(p = 0.766). And this score showed a tendency to improve with passage of time in the 1-minute, 3-minutes, and 10-minutes PI groups (p = 0.012, p = 0.001, p < 0.001 by Friedman's test) (Figure 4).

5. Light microscopic examination of conjunctival tissue

Conjunctival cells prepared on day 7 were observed by light microscopy. Abundant goblet cells and multiple layers of epithelial cells were apparent in the control and 1-minute PI group. However, the goblet cell counts decreased, and single layers of epithelial cells became more prevalent as the PI exposure time increased to more than 3 minutes (Figure 3). These findings were similar to those obtained upon conjunctival impression cytology test.

6. Immunofluorescence staining for MUC5AC of conjunctival tissue

Goblet cells were observed by immunofluorescence staining using an anti-MUC5AC antibody. Abundant MUC5AC was present in the conjunctival epithelia of the control and 1-minute group. However, the extent of MUC5AC staining was reduced markedly in the 3-minutes and 10-minutes PI groups compared to the other groups (Figure 3).

7. Transmission electron microscopy (TEM) of conjunctival tissue

Under the transmission electron microscope (TEM), abundant microvillar structures were evident on the surfaces of conjunctival epithelial cells of the control and 1-minute PI group. In addition, goblet cells with many secretory granules, which play an important role in synthesis of MUC5AC, were observed (Figure 5a, 5b). However in both the 3-minutes and 10-minutes PI groups, the extent of microvillar structure decreased, and apoptotic morphological changes including nuclear fragmentation, condensation and peripheral migration of chromatin were evident in conjunctival epithelial cells (Figure 5c, 5d). Such ultrastructural disturbances were more severe in the 10-minutes PI group (Figure 5d).

8. Scanning electron microscopy (SEM) of corneal tissues

Scanning electron microscopy (SEM) showed that the superficial cells were intact with normal microvilli in the control group (Figure 5e). However, SEM images showed corneal epithelial toxicity that the

microvilli seemed to be injured in the all three PI groups. The longer the exposure time, the greater was the reduction in the number of microvilli (Figure 5f, 5g, 5h).

Discussion

Many studies have explored ocular surface damage associated with cataract surgery.[1, 2, 4–6, 16–20] Some factors are well-known to be associated with postoperative dry eye. These include corneal damage caused by ultrasound used during cataract surgery, topical anesthesia, eye drops containing preservatives such as benzalkonium chloride, corneal desensitization caused by corneal incision, surgical trauma, inflammatory responses producing chemicals such as free oxygen radicals, and exposure to light from microscopes.[1, 2, 4–6, 16–20] In clinical practice, some ophthalmologists have detected corneal damage immediately after preoperative PI instillation. Thus, we hypothesized that PI instillation might be pathogenic factor of dry eye syndrome in terms of inducing ocular surface damage in patients undergoing ophthalmic surgery. The purpose of our study was to evaluate the effects of 5% (w/v) PI on the ocular surfaces and identify possible pathogenic factor triggering ocular surface damage after cataract surgery.

Povidone-iodine (PI) is bactericidal against a wide range of bacteria and is also effective to kill fungi, protozoa, and viruses.[21–23] When the PI complex reaches the cell wall, free iodine is released and is rapidly cytotoxic, killing prokaryotic cells within 10 seconds. So in the 2007 ESCRS multicenter study on prophylaxis for endophthalmitis, application of one drop of 5% (w/v) PI or 10 mL of this solution on a sponge pad to the cornea and the conjunctival sac for a minimum of 3 minutes was recommended for preoperative antisepsis of the cornea and conjunctiva.[7] But PI also has potentially both cytotoxic and cytocidal effect for mammalian cells because the action of the material is nonspecific, attributable to oxidization of free iodine in aqueous solution.[24] Although a 5% (w/v) PI solution is thought to be safe and effective antiseptic when used on skin and mucous membranes, the results of our present study have shown that PI can induce ocular surface damages. We also evaluated the effects of 5% (w/v) PI applied for various exposure times, after single instillation onto the ocular surface of normal rabbits, by performing the Schirmer test, Rose Bengal staining, corneal fluorescein staining, conjunctival impression cytology and biopsy. We compared the changes of ocular surface among control group and study groups exposed to 5% (w/v) PI for 1, 3, and 10 minutes. The Rose Bengal staining scores were significantly increased immediately after PI instillation as the PI exposure time increased although the Schirmer test results were not different among the four groups. And on conjunctival impression cytology, the GCD was decreased and the Nelson score was increased with increasing exposure time. The GCD and Nelson score were significantly different between the 1-minute and 3-minutes PI group and the 1-minute and 10-minutes PI group. Conjunctival histology performed on day 7 revealed similar results. The conjunctival epithelial region of MUC5AC staining was reduced markedly in the 3-minutes and 10-minutes PI groups compared to the other groups. And SEM-based histological analyses showed loss of microplicae of superficial cornea. This corneal epithelial cell damage is also the reason for the positive staining in the fluorescein. We noted that exposure time-dependent pathophysiological ocular surface changes were similar to those seen in the dry eye syndrome; these included the reduction of GCD, an increase of

conjunctival epithelial cell size, squamous metaplasia of conjunctiva and damaged superficial layer of the cornea.

The mucin and the microplicae play a role in tear film adhesion and stabilization to the corneal surface. And the microplicae are also important for the attachment of mucin to the cornea. Decreased production of MUC5AC, as well as changes in membrane-associated mucins also leads to loss of microplicae.[25–28] The functions of mucous layer play a vital role in the stability of the tear film, converting the hydrophobic corneal epithelium to be hydrophilic and lubricating the ocular and palpebral surfaces. Thus, the damages of microplicae and decreased mucin production induced by PI interfere the formation of the innermost mucous layer of the tear film, and would lead to less retention of fluid even with functional lacrimal glands.

It is important to define the ocular surface toxicity caused by PI because of the recent spotlight cast on ocular surgery-associated dry eye syndrome. PI has been reported to be cytotoxic to the eye. Jiang et al. found that severe corneal epithelial damages were developed after PI instillation into the conjunctival sac, and significant corneal edema was observed after PI injection into the anterior chamber.[3] MacCrae et al. studied rabbit corneas after PI application, and noted moderate transient corneal edema at 5 minutes, which was resolved 3 hours later.[12] Whitacre and Crockett assessed the toxicity of intravitreal PI by injecting 0.1 mL amounts of PI solutions at 0.05%, 0.5%, and 5% (all w/v) into the vitreous cavity.[29] One of 10 eyes injected with 0.05% PI had iritis, intraretinal hemorrhage and mild retinal necrosis, whereas all four eyes injected with 5% (w/v) PI had dense cataracts and full-thickness retinal necrosis. Apart from the direct toxicity of topical PI for the ocular surface, both contact dermatitis and keratoconjunctivitis sicca have been (rarely) reported.[21, 30] Therefore, intraocular contamination with PI is of concern. To the best of our knowledge, few studies have addressed the cytotoxicity of PI for the ocular surface, especially the effect of PI on conjunctival goblet cell function. And unfortunately, PI exposure times were not compared; all data were compared to only those of the control group. Although ocular toxicity of PI may increase as the exposure time becomes longer, few studies have sought to clearly define an appropriate exposure time.

It is clear that, in vivo, periocular preparation with PI alone (even for a long time) may not completely eradicate all types of microorganism that give rise to postoperative endophthalmitis. And as the results of present study show, the longer the PI exposure time, the more severe the ocular surface damages, so in order to minimize ocular surface toxicity, it would be better to use PI an optimum exposure time along with other prophylactic antibiotics or bactericidal agents that supplement the disinfective effect. In our present study, PI exposure for 1 minute was not associated with any microscopically apparent damage to conjunctival epithelial cells, although corneal and conjunctival epithelial cells that were no longer adequately protected by tear films stained with Rose Bengal to a greater extent than control group. Upon conjunctival impression cytology, it was remarkable that no significant difference was apparent between the control and 1-minute PI group. Thus, if the antimicrobial effect of 1 minute of PI exposure is as effective as that afforded by 3 minutes of PI exposure, the optimum PI exposure time affording

maximum eradication of microorganisms capable of causing endophthalmitis, but without causing ocular surface toxicity, should be 1 minute rather than the 3 minutes of the previous guidelines.

The present study had several limitations. The follow-up period (7 days) was short, and the sample size was small, meaning that possible reversibility of ocular surface toxicity was not observed. The GCD of the 3-minutes group tended to recover on day 7. Our results indicate that long-term instillation of PI causes acute injury to the ocular surface and may cause chronic injury or induce dry eye. Nevertheless, as the 10-minutes group, unlike the 3-minutes group, showed no signs of recovery, further study is required to determine the possibility of chronic conjunctival injury following long-term PI instillation, to explore whether and when damaged ocular surfaces can recover. Unfortunately, we could not check tear break up time for technical reasons and equipment-related problems. Although we could not demonstrate whether the decrease in GCD and MUC5AC directly caused dry eye, one study suggested a correlation between MUC5AC expression and dry eye clinical test results such as tear break up time.[31] Expression of conjunctival MUC5AC and the squamous metaplasia in CIC are closely correlated with tear break up time as a dry eye severity indicator.[31, 32] Therefore, based on these studies, we consider that the laboratory tests implemented in our study might supersede tear break up time. Use of PI may be a pathogenic factor causing postoperative dry eye resulting from the decrease in GCD and MUC5AC stain. However, we will measure tear break up time for confirmation in our next human clinical trial.

Conclusions

In conclusion, we have shown that longer exposure times with 5% (w/v) PI increasingly damaged the cornea and conjunctiva of normal rabbits (as shown by Rose Bengal staining and fluorescein staining); decreased the GCD, and triggered development of squamous metaplasia, as revealed by impression cytology and corneal and conjunctival histology. We could not confirm whether PI toxicity triggers not only acute injury but also chronic injury. So, further clinical study is required to clarify the relationship between PI and ocular surface toxicity. And also, we evaluated that such an instillation of PI might be pathogenic in terms of inducing ocular surface damage in patients undergoing ophthalmic surgery. Therefore, the toxicities of PI to ocular surface could be a possible pathogenic factor triggering dry eye syndrome after ophthalmic surgery.

Abbreviations

CIC: Conjunctival impression cytology, GCD: Goblet cell density, MUC5AC: Mucin 5 subtype AC, PAS: Periodic acid–Schiff, PBS: Phosphate-buffered saline, PI: Povidone-iodine

SEM: Scanning electron microscopy, TEM: Transmission electron microscope

Declarations

Ethics approval and consent to participate

All experimental procedures were performed according to the guidelines of the Association for Research in Vision and Ophthalmology (ARVO) as set out in the “Statement for the Use of Animals in Ophthalmic and Visual Research” and the ARRIVE Guidelines for reporting animal research, and approved by the institutional animal care and use committee of Catholic university of Korea.

Consent for publication

All authors were given the consent for publication of this manuscript.

Availability of data and material

All data are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

SYK, YSA and HSK participated in the design of this study, SYK carried out the study, collected the data and performed the statistical analysis. YSA and YJL drafted the manuscript. HSK performed manuscript review. All authors read and approved the final manuscript.

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Figures

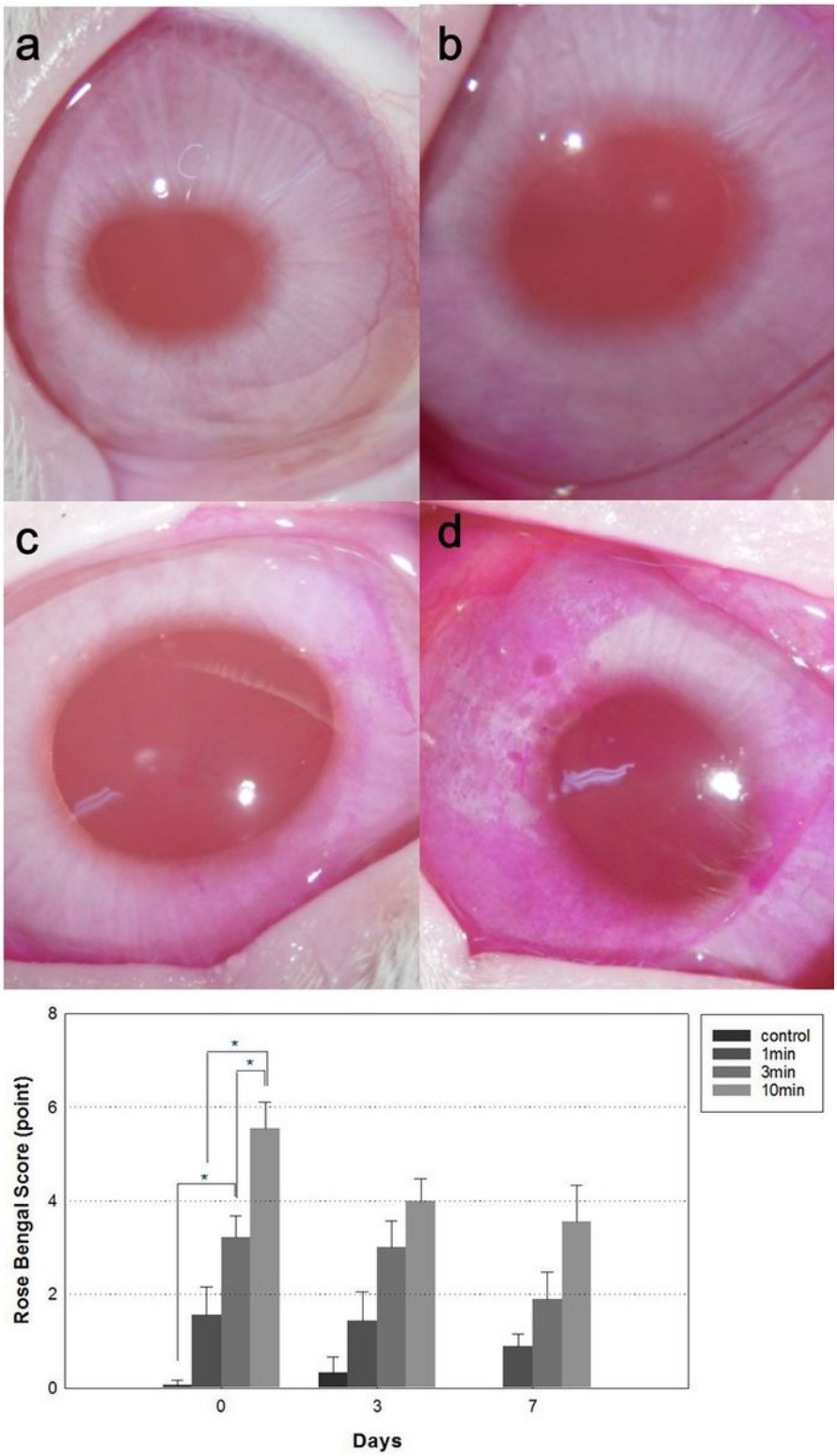

Figure 1

(above) Representative image samples stained with Rose Bengal in each group performed on day 0 (immediately after povidone-iodine (PI) treatment) (a) control group (b) 1-minute PI group (c) 3-minutes PI group (d) 10-minutes PI group. (below) Comparison of Rose Bengal staining scores among the four groups. The Rose Bengal staining scores differed significantly between the control and 3-minutes PI group, the 3-minutes and 10-minutes PI groups, and the 1-minute and 10-minutes PI groups on day 0.

Data show means ± standard deviations (error bars). Day 0 indicates the day immediately after PI instillation. * $p < 0.05$ (Steel–Dwass test for the differences among the four groups).

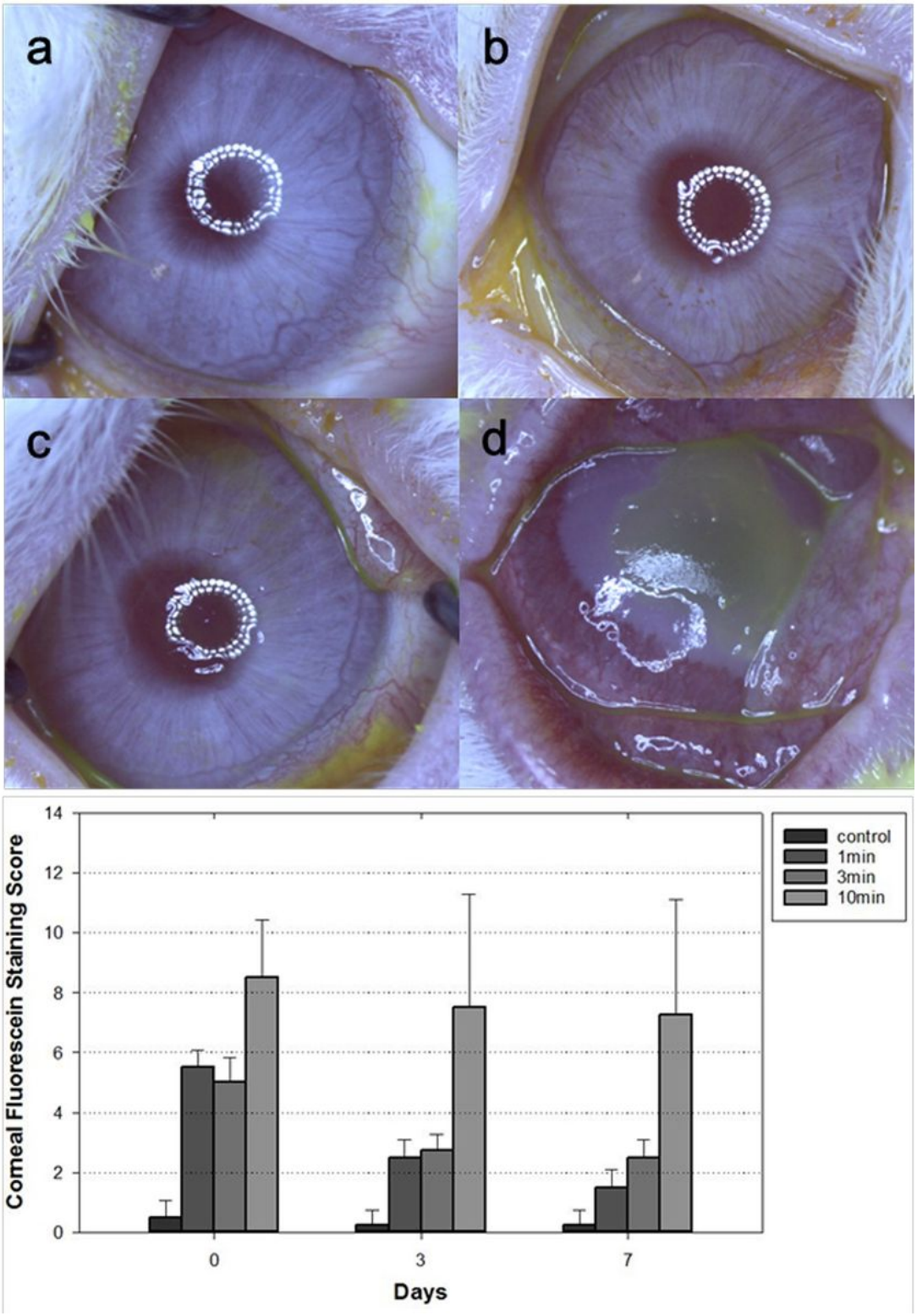

Figure 2

above) Representative image samples stained with fluorescein in each group performed on day 7 (a) control group (b) 1-minute PI group (c) 3-minutes PI group (d) 10-minutes PI group. (below) Comparison of corneal fluorescein staining scores among the four groups. Figure 2b and 2c shows micropunctate or

macropunctate staining of cornea in the 1-minute and the 3-minutes PI groups. The 10-minutes PI group shows large epithelial defect on superficial cornea (d). No significant differences in the corneal fluorescein staining score at each time point (Steel–Dwass test for the differences among the four groups). Data show means ± standard deviations (error bars). Day 0 indicates the day immediately after PI instillation.

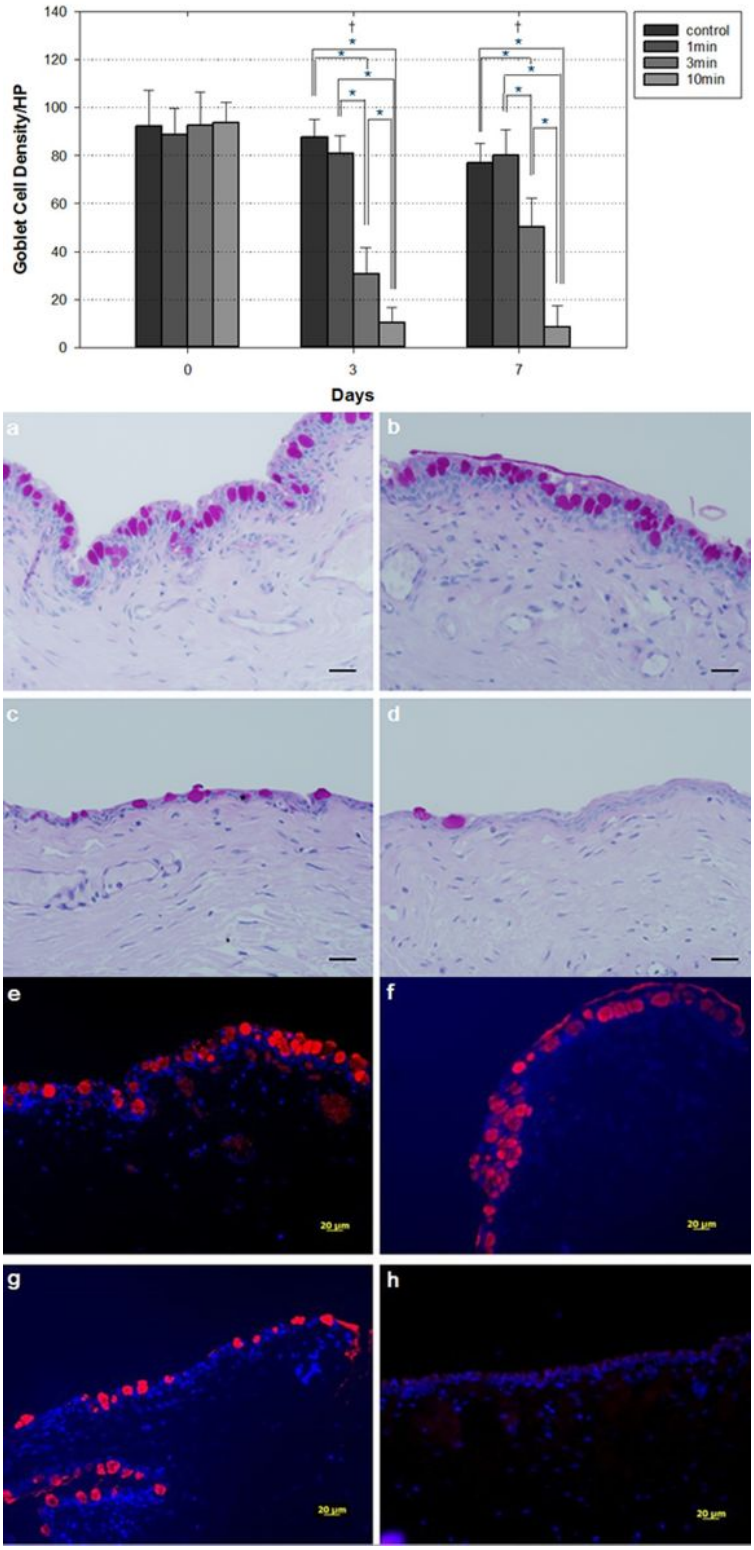

Figure 3

(top) Comparison of goblet cell density (GCD) among the four groups. Significant differences were noted between the control and 3-minutes PI group, the control and 10-minutes PI group, the 1-minute and 3-minutes PI group, the 1-minute and 10-minutes PI group, and the 3-minutes and 10-minutes PI group on day 3 and 7. Data are the means ± SDs (error bars). Day 0 indicates the day immediately after PI instillation. * $p < 0.05$ (Tukey–Kramer test for the differences among the four groups). (middle) Representative images obtained on histological examination of conjunctiva on day 7. (a) In the control group, the conjunctiva is composed of a single cuboidal basal cell layer and three-to-four layers of epithelial cells, with numerous interspersed goblet cells. (b) In the 1-minute PI group, the conjunctival epithelium and goblet cell density are almost normal. (c) In the 3-minutes PI group, the conjunctival epithelium is thinner and the cuboidal basal cell layer is lost. The extent of the area stained by periodic acid-Schiff is reduced. (d) In the 10-minutes PI group, the conjunctival epithelium is even thinner and goblet cell numbers are decreased markedly. (Bar : 20 μm) (bottom) Representative images obtained on immunofluorescence staining of the conjunctiva on day 7. MUC5AC staining (red) was performed, and Hoechst 33342 was used for nuclear counterstaining (blue). (e) Many control group cells were detected in the anti-MUC5AC antibody conjunctiva stain. (f) Many cells also stained in the 1-minute PI group. (g) The number of goblet cells stained by the anti-MUC5AC antibody decreased in the 3-minutes PI group. (f) No MUC5AC-positive cells are observed in the 10-minutes PI group. (Bar : 20 μm)

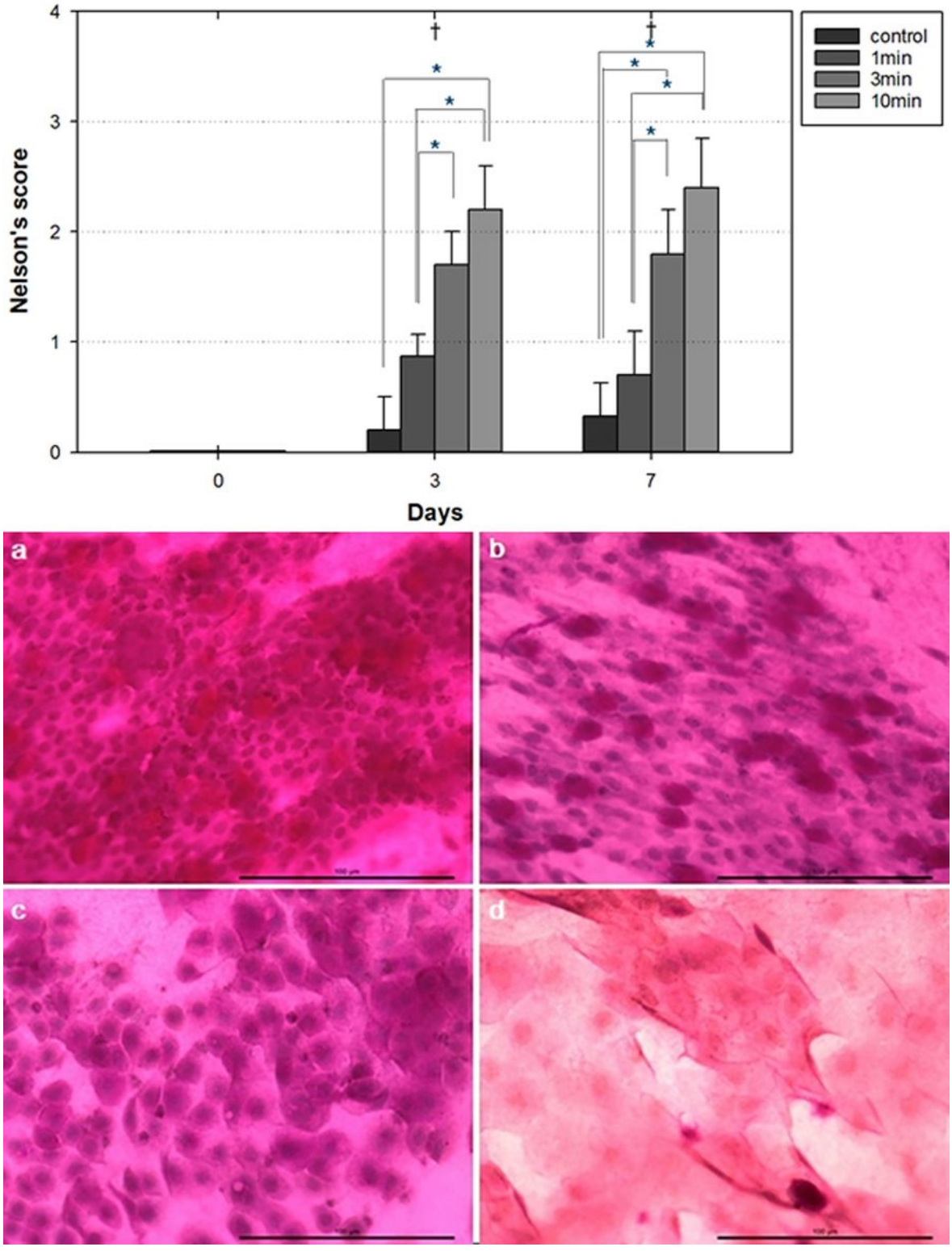

Figure 4

(above) Comparison of the severity of squamous metaplasia according to Nelson grading system among the four groups. Significant differences were noted between the control and 10-minutes PI group, the 1-minute and 3-minutes PI group and the 1-minute and 10-minutes PI group on day 3, and between the control and 3-minutes PI group, the control and 10-minutes PI group, the 1-minute and 3-minutes PI group and the 1-minute and 10-minutes PI group on day 7. Data show means ± SDs (the error bars). Day 0

indicates the day immediately after povidone-iodine instillation. * $p < 0.05$ (Steel–Dwass test for the differences among the three PI groups). (below) Representative images from conjunctival impression cytology of each group performed on day 7. (a) In the control group, the conjunctival impression cytological grade is 0. The epithelial cells are small and rounded. The nuclei are large, and stained with hematoxylin, at a nucleocytoplasmic ratio of 1:2. The abundant goblet cells are plump and oval with an intensely PAS-positive cytoplasm (400X). (b) In the 1-minute PI group, the cytology grade is 0-1. The epithelial cells are slightly larger than control cells and more polygonal in shape. Goblet cells are abundant. (c) In the 3-minutes PI groups, the cytology grade is 2-3. The epithelial cells are slightly larger and more polygonal in shape. The nuclei are smaller, and have a nucleocytoplasmic ratio of between 1:2 and 1:3. Goblet cells numbers are markedly decreased. (d) In the 10-minutes PI group, the epithelial cells are larger and more polygonal. The nuclei are smaller and have a nucleocytoplasmic ratio of between 1:4 and 1:5. Goblet cells are scanty. Bar: 100 μm.

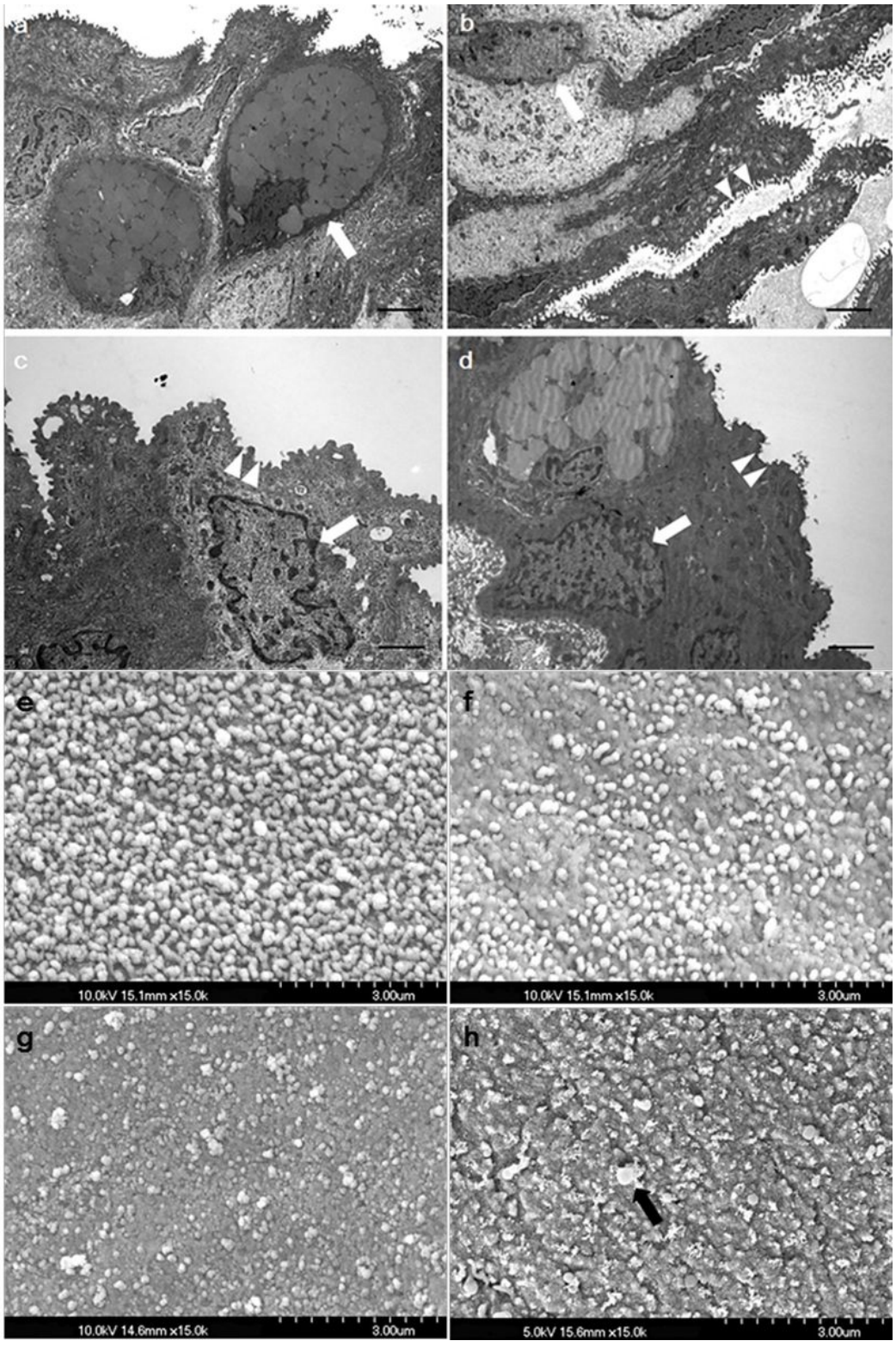

Figure 5

(a-d) Representative transmission electron microscopy (TEM) images of the conjunctiva and (e-h) scanning electron microscopy (SEM) images of the cornea on day 7. a,e: control group, b,f: 1-minute PI group, c,g: 3-minutes PI group, d,h: 10-minutes PI group. TEM images revealing the conjunctival epithelial ultrastructure(a-d). Abundant microvillar structures on the surfaces of conjunctival epithelial cells(arrowhead) and goblet cells with many secretory granules(arrow) are observed in the control and 1-

minute PI group(a, b), whereas the extent of microvillar structure decreased, and apoptotic morphological changes (including nuclear fragmentation , condensation, and peripheral migration of chromatin) were shown in the other groups(c,d). (Bar : 2 μm). SEM images revealing the corneal epithelial structure(e-h). The superficial cells were intact with normal microvilli in the control group(e), whereas the flat surfaces with destroyed microvilli were shown in the all three PI groups. The longer the exposure time, the greater was the reduction in the number of microvilli(black arrow)(f-h).