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ORIGINAL ARTICLE

Comparison of the lubricant eyedrops Optive®, Vismed Multi®, and Cationorm® on the corneal healing process in an ex vivo model

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ABSTRACT

Purpose: To evaluate the impact of lubricant eyedrops on the corneal healing process and corneal toxicity.
Methods: Optive® and Cationorm® were tested regarding corneal irritability against Vismed Multi® and 0.01% benzalkonium chloride as negative and positive control, respectively. Formulas were applied on rabbit corneas (n = 5) cultured on artificial anterior chambers (EVEIT system) hourly over 3 days. Initially, 4 corneal abrasions (2-5.4 mm²) were induced. All defects were monitored during drug application by fluorescein stains and photographs. To ensure corneal vitality, glucose and lactate concentrations were determined photometrically in artificial anterior chamber fluids. Corneal fluorescein sodium permeability was tested as an indicator of the corneal barrier function.
Results: Optive® and Vismed Multi® showed a complete corneal healing on day 2. In one cornea (Optive®), erosion reoccurred on day 3. Erosion sizes of Cationorm®-treated corneas increased significantly from 12.20 mm² to a subtotal erosion of 51.89 mm² on day 3. Histology revealed epithelial loss and severe alterations of the superficial stroma for Cationorm®. Glucose and lactate concentrations did not change after application of Optive® and Vismed Multi®. In contrast, Cationorm®- and BAC-treated corneas showed a significant increase in lactate concentrations.
Conclusions: Vismed Multi® application resulted in rapid corneal healing. Whether the toxicity seen for Optive® in one cornea is a valid result should be examined further. Cationorm® showed considerable corneal toxicity that could be caused by its additive, cetalkonium chloride. Otherwise, the electrostatic properties of Cationorm® led to a drug film on the area of epithelial loss that could hinder epithelial cell migration and adhesion in order to heal the lesion.

Keywords: Cationorm®, EVEIT, Ex vivo model, Lubricant eyedrops, Optive®, Vismed Multi®

Introduction

Dry eye syndrome (DES) is recognized as a growing public health problem and one of the most frequent reasons for affected individuals to seek ophthalmologic intervention. Dry eye syndrome can be defined as a multifactorial disease of the tears and ocular surface that results in symptoms of discomfort, visual disturbance, and tear film instability, with potential damage to the ocular surface (1). Physiologically, the ocular surface is protected by the tear film, consisting of an aqueous, lipid, and mucous layer. It is believed that any decrease in tear volume either by reduced secretion or a change in its composition and, thus, increased tear evaporation causes DES. The basal tear secretion is regulated by complex pathways. On the one hand, neurons consisting of afferent sensory nerves in the cornea and conjunctiva stimulate efferent parasympathetic and sympathetic nerves that innervate the lacrimal gland (2). On the other hand, tear secretion is regulated by a specific protein content of the ocular surface, which is yet not well understood. A known regulatory protein is the prosecretory tear protein lacritin that has been shown to cause a decreased incidence of DES (3).

Evaporative DES, which accounts for about 50% of DES cases, is supposedly caused by dysfunction of meibomian glands, in particular, because meibomian oil forms the lipid layer at the surface of the tear film, thereby preventing excessive evaporation (4, 5). Besides these mechanisms, such factors as age, sex, diet, and the environment also play a role in the pathophysiology of DES (6).

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Applying lubricant eyedrops is the conventional way to treat DES and corneal erosions (7). To enhance the retention time of fluids on the ocular surface, artificial tear products are formulated with viscous agents or lubricants such as hyaluronic acid, polyvinyl alcohol, polyethylene glycol 400, propylene glycol, glycerin, and dextran (8, 9). As a low pH value and a high tear osmolality are major factors to trigger inflammation in DES, lubricants are often formulated with pH buffers and as hypo-osmolar solutions (10). Since these products mainly target the hyposecretory form of DES, innovative products have also been designed to replace lipids in order to prevent tear evaporation as well. Systane Balance® (Alcon, Fort Worth, TX, USA), for example, is a novel formulation that contains both polymers and lipids to build up an aqueous and lipid phase, respectively (11). Comparable formulations are Soothe® (Bausch & Lomb, Rochester, NY, USA), containing mineral oil, and Optive® (Allergan, Irvine, CA, USA), containing hygroscopic glycerin. More recently, Cationorm® (Novagali, Evry, France) was brought to market using the Novasorb® technology platform. This positively charged nanoemulsion is believed to bind to negatively charged glycosyl aminoglycans lining the ocular surface (12), thereby prolonging the residence time of the formulation on the ocular surface. In addition, the oil droplets are nano-sized, which creates a huge contact surface (13).

Little attention has been paid to possible side effects of these new products. The aim of this study was to compare the corneal healing efficiency and possible corneal side effects of 2 novel formulations, Cationorm® and Optive®, in comparison with those of a conventional hyaluronic acid-based formulation, Vismed Multi® (TRB Chemedica, Geneva, Switzerland). The Ex Vivo Eye Irritation Test (EVEIT) system was used as a model to provide irritated corneas in order to test the interference of corneal healing with novel artificial tear products (14, 15).

Materials and Methods

Test substances

Two new artificial tear products were tested: Optive® (Allergan) and Cationorm® (Novagali). Optive® consists of the lubricants carboxymethylcellulose sodium (0.5%) and glycerin (0.9%), the sugar erythritol, l-carnitine, and the preservative stabilized oxychloro complex or Purite®.

Cationorm® is a mixture of the lubricants glycerol and paraffin, the surfactant tyloxapol and co-surfactant poloxamer 188, the buffers trometamol and trometamol hydrochloride, and the preservative cetalkonium chloride (CKC).

These artificial tear products were tested against Vismed Multi® (TRB Chemedica), containing hyaluronic acid and electrolytes, as negative control, and the preservative benzalkonium chloride (BAC), as positive control. To avoid any loss of substance due to evaporation of the test solvents, all test substances were applied directly by pipetting a volume of 30-50 µL onto the corneal vertexes.

All substances were applied hourly over 3 days.

EVEIT

To study the corneal healing process and corneal drug toxicity, the EVEIT (Fig. 1) was employed. The EVEIT system is a nonanimal consuming test that simulates the anterior ocular chamber with a physiologic corneal barrier for testing corneal drug permeation and corneal toxicity. This test has been described in detail previously (13, 14). Briefly, the EVEIT system consists of a culture of rabbit corneas obtained from slaughterhouse rabbits used for human food supply. The eyes are separated and the corneas excised and placed in an artificial anterior ocular chamber for long-term nutrition. The rabbit corneas are prepared and cultivated within 8 hours postmortem. For nutrition, the chamber is supplied with a culture medium containing Earle salts and HEPES buffer (Eagle minimal essential medium [MEM], HEPES buffer 5.8 g/L).

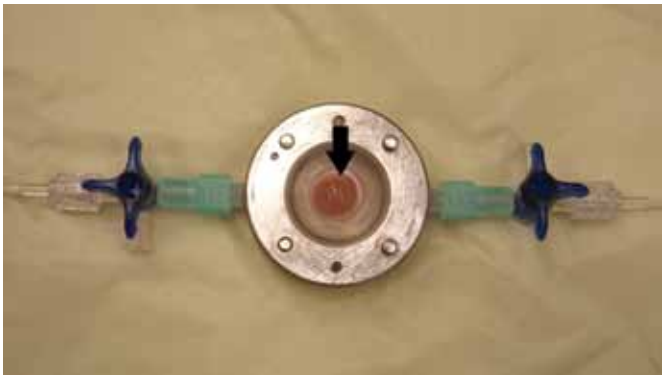

Fig. 1 - The Ex Vivo Eye Irritation Test system. A rabbit cornea (arrow) is centered on an artificial anterior chamber supplied by a constant flow of a medium solution.

In these experiments, the medium was constantly replenished by a micropump (Ismatec IPC, IDEX Health & Science GmbH, Wertheim, Germany) with an entrance pH value of 7.4 ± 0.2 and a flow rate of 6.44 µL/min, which imitates the physiologic conditions in the eye. Five corneas per substance were used in the experiments except for BAC (n = 1). The corneas were incubated at a temperature of 32°C and a humidity of more than 95% throughout all the experiments. There was no additional moisturizing with culture medium MEM. All the experiments were performed in accordance with the Code of Ethics of the World Medical Association.

Corneal abrasion

After 24 hours of stabilization within the EVEIT culturing system, the corneas were evaluated by microscopy (Fig. 2). Only those corneas with an intact epithelium and without opacities were used for further experiments. Therefore, the integrity of both the epithelial and endothelial sides was monitored using a phase-contrast microscope-integrated camera (KY-F1030U, JVC, Bad Vilbel, Germany) mounted on a Z16 APO microscope (Wetzlar, Germany) connected to DISKUS software (Hilgers, Koenigswinter, Germany).

Before corneal healing experiments started, corneal erosions measuring 2-5.4 mm² were induced by an abrasive cornea drill, which was placed on the cornea in a square pattern. Defect sizes were monitored by fluorescein sodium stains (0.17% aqueous solution), with yellow-green fluorescence indicating the areas of epithelial defects. In order to measure, the erosions were circumscribed using a software tool of the

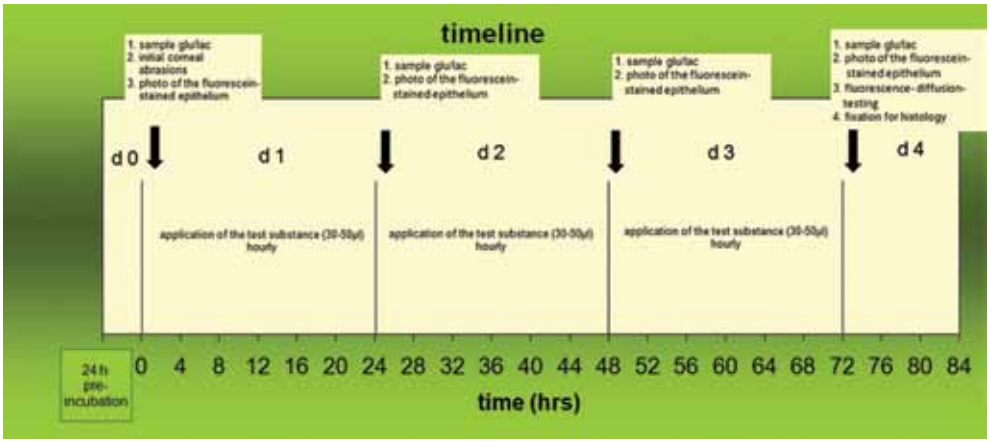

Fig. 2 - Experimental procedure presented as a timeline over 4 days. The drug application regimen, time points of photodocumentation, corneal erosion induction, fluid sampling, and histology (↓) are displayed.

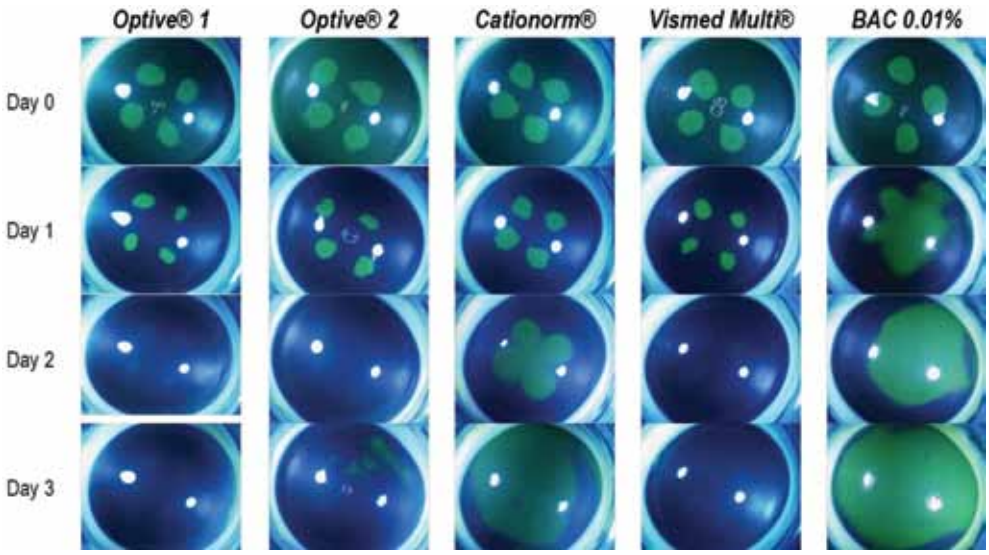

Fig. 3 - Representative microscopic images of the healing process of the corneal epithelium under drug application. Initially, 4 small corneal abrasions (2-5.4 mm²) were generated (day 0). The effect of the drugs Optive®, Cationorm®, Vismed Multi®, and 0.01% benzalkonium chloride (BAC) on the corneal erosion size within 3 days of drug application are displayed. For Optive®, 1 out of 5 corneas (Optive® 1) is shown where the epithelium is healed at day 2 and a second cornea where erosions reappeared at day 3 (Optive® 2).

microscope-integrated camera (KY-F1030U, JVC) mounted on a Z16 APO microscope connected to DISKUS software (Hilgers, Koenigswinter, Germany). Erosion sizes are given in square millimeters.

Toxicity assessment

Corneal metabolic activity. Corneal vitality was assessed by demonstrating metabolic activity. Therefore, the concentrations of glucose (GOD-PAP, Greiner Diagnostic GmbH, Bahlingen, Germany) and lactate (LOD-PAP, Greiner Diagnostic GmbH) were quantified photometrically (Fluostar Optima microplate reader, BMG Labtech GmbH, Offenburg, Germany) in the eluted medium of the anterior chamber after bypassing the corneal endothelium. The glucose/lactate concentrations were analyzed daily.

Corneal barrier function. To determine how and to what extent drugs influence the overall corneal barrier function, substance permeation experiments were conducted. Fluorescein sodium solution was chosen since it can be easily detected in the EVEIT anterior chamber fluid by photometry (Fluostar Optima microplate reader). Five corneas for each drug tested were incubated with apically applied aqueous fluorescein sodium solution (5 mg/mL, 100 µL each cornea). Samples were taken before (day 0) and after the experimental time at day 3.

Histology. Corneal morphology was evaluated histologically using a standard hematoxylin & eosin staining method.

Results

The corneal healing process and metabolic activity under test substance application

Within 3 days of test substance application, corneal erosion sizes varied substantially depending on the test substance applied (Figs. 3 and 4). Vismed Multi®, as negative control, started on corneas showing an average corneal lesion size of 12.23 mm² and that healed completely on day 2 ($p<0.001$). A comparable pattern was observed for Optive® ($p<0.001$). Yet a resurgence of erosion at day 3 could be observed for a single corneal preparation exposed to Optive® (Fig. 3).

For corneas treated with Cationorm®, the area of mechanical erosion started at 12.20 mm². Initially, a decrease in erosion size could be observed. After 3 days of drug application, however, the corneal erosions enlarged significantly ($p = 0.025$) to 51.89 mm². Even more severe damage was caused

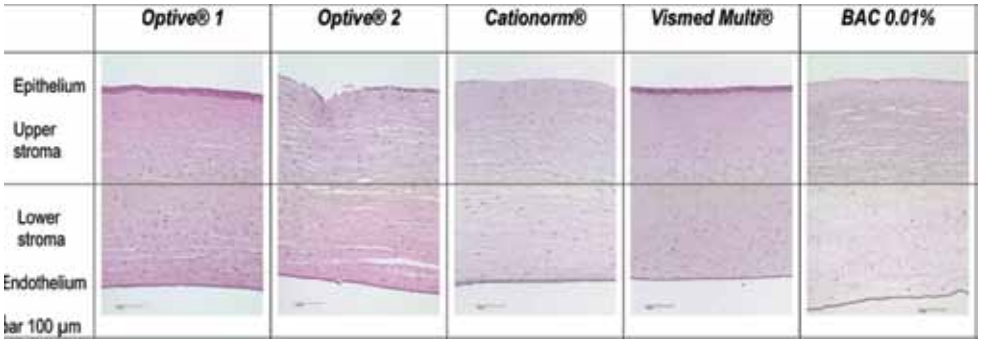

Fig. 4 - Illustration of the corneal healing process under drug application. The mean corneal erosion sizes in mm^2 are plotted against time (experimental days) for Optive®, Cationorm®, Vismed Multi®, and benzalkonium chloride (BAC) treated corneas.

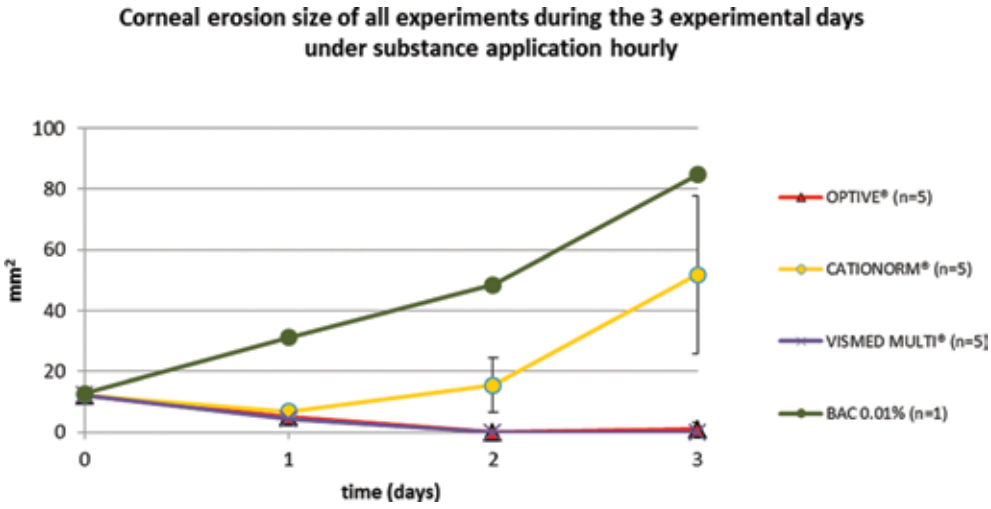

Fig. 5 - Representative micrographs of hematoxylin & eosin stained cornea after Cationorm®, Optive®, benzalkonium chloride (BAC), or Vismed Multi® were applied for 3 days.

by the chemical BAC 0.01%. Here, the erosion size increased from 12.75 mm^2 to 84.85 mm^2 (n = 1) after a 3-day exposure.

Histology

Regarding the corneal microstructure at the end of experiments on day 3, histology revealed complete healing of the epithelial layer, dense stroma, and regularly arranged keratocytes for Vismed Multi®. Both the Descemet membrane and the endothelial layer were present without any damage in structure (Fig. 5).

For corneas exposed to the test substance Optive®, histology revealed a perfect healing of the epithelial layer with closed and multilayered epithelial cells. The upper stroma showed a loss of keratocytes. A diffuse swelling of the collagen layer was evident in the whole stroma. The Descemet membrane and endothelial layer were present without any defects in structure. By contrast, one cornea displayed severe alteration of the superficial cornea, defects in the epithelial layer, and a loss of keratocytes underneath the area of erosion. In addition, a diffuse swelling of the stromal collagen layer was seen.

For both Cationorm® and BAC, the corneal epithelial layer was almost completely lost. The number of stromal keratocytes was greatly reduced, while the corneal endothelium and Descemet membrane were normally structured. These effects were more severe for BAC 0.01% than for Cationorm® (Fig. 5), indicated by a massive diffuse corneal edema for BAC.

Corneal metabolism

As an indicator of metabolic stress caused by topical drug application, glucose and lactate concentrations in the outflow medium of the artificial anterior chamber were analyzed. Figure 6 displays the glucose (Fig. 6a) and lactate (Fig. 6b) concentration for each drug before, during, and after drug application. Here, there was no observed change in lactate/glucose concentrations for Vismed Multi® treated corneas between baseline on day 0 until the end of drug application on day 3 (lactate p = 0.2768, glucose p = 0.646). Comparably, no significant changes were observed for Optive® (lactate p = 0.056, glucose p = 0.645). In contrast, Cationorm® application led to a significant decrease (p = 0.047) in glucose and an increase in lactate concentrations (p = 0.00991). As for Cationorm®, BAC (n = 1) induced an increase in lactate from 2.51 to 6.4 mmol/L (for Cationorm® 3.62 ± 0.15 to 5.55 ± 1.27 mmol/L), whereas glucose concentrations remained unchanged.

The corneal barrier function after test substance application

Corneal fluorescein sodium permeability testing is an indicator for the integrity of the corneal barrier function. Figure 7 compares the fluorescein sodium concentration in the artificial anterior chamber medium before and after 3 experiment days of hourly exposure to the test substances. With Vismed Multi® (p = 0.97) and Optive® (p = 0.38), no significant change in corneal drug permeability is detected on day 3 in comparison

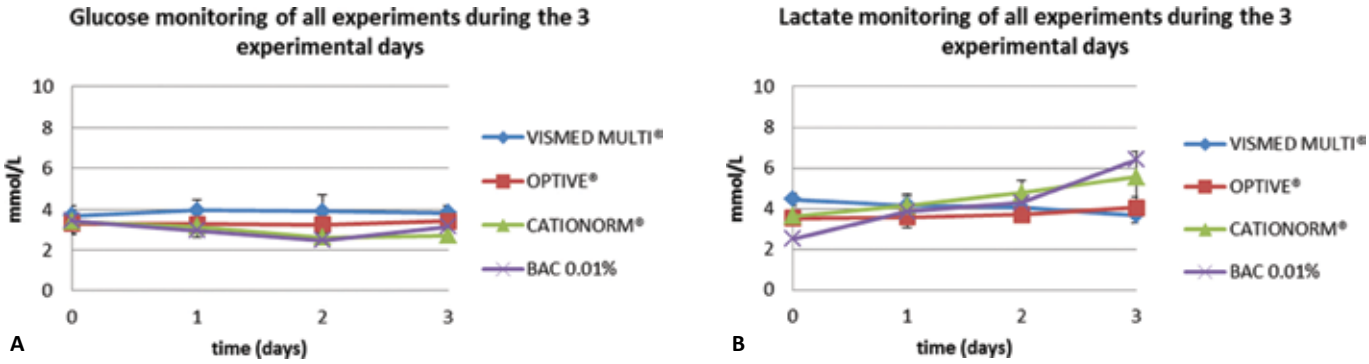

Fig. 6 - (A) Comparison of the glucose concentration in cornea exposed to Cationorm®, 0.01% benzalkonium chloride (BAC), Optive®, or Vismed Multi®. A decrease in the glucose concentration was found initially for Cationorm® and BAC. On the other hand, glucose concentrations were stable for Optive® and Vismed Multi®. **(B)** Corneal metabolic activity indicates an increase in the lactate concentration for Cationorm® and BAC. As expected, lactate concentrations were stable for Optive® and Vismed Multi®.

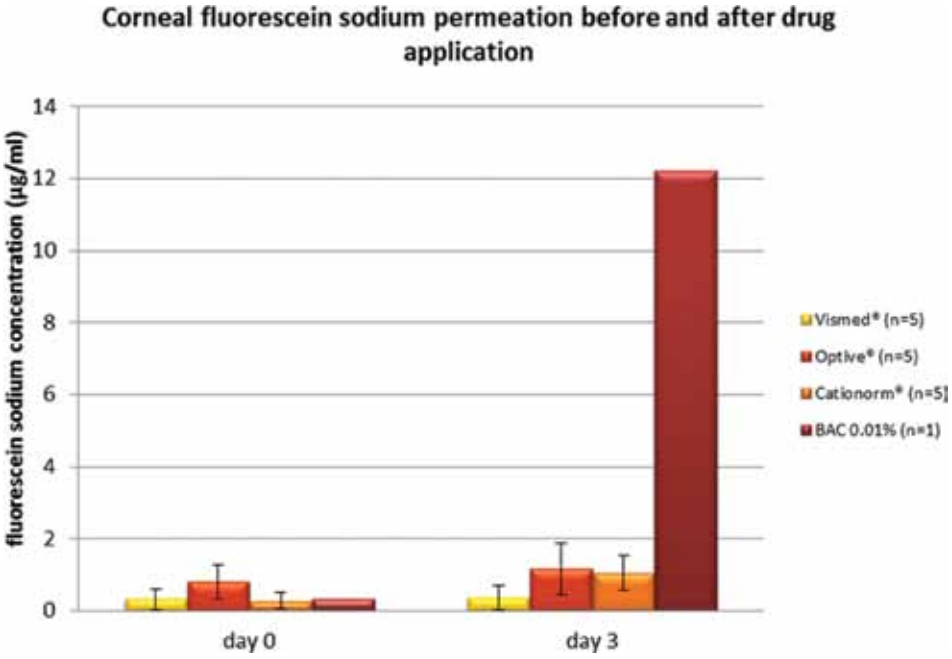

Fig. 7 - Corneal permeability of all corneas is illustrated. Tests were carried out on day 0 and day 3 (after hourly exposure to the test substances). An unpreserved solution of sodium fluorescein (5 mg/mL) was applied on the apex of the corneas, and the fluorescein concentration was measured in the perfusion medium of the anterior chamber via photometry after 60 minutes. Cationorm® increased the corneal permeation of fluorescein significantly ($p = 0.021$). A noticeable increase was also observed with 0.01% benzalkonium chloride (BAC) ($n = 1$).

to baseline values. Cationorm®, however, shows a significant increase ($p = 0.005$) in fluorescein sodium concentration in the anterior chamber medium indicating a disruption of the corneal barrier function induced by Cationorm®. Similarly, even a 10 times higher anterior chamber fluorescein sodium concentration is found for BAC compared to Cationorm®.

Discussion

The major goal in treating DES is to improve the quantity and quality of the tear film to protect the ocular surface. This study investigated the novel lipid-water emulsion-based lubricants Cationorm® (Novagali, CKC preserved) and Optive® (Allergan, Purite® preserved) in comparison to hyaluronic acid-based Vismed Multi®. As a positive control, 0.01% BAC, a preservative known for its corneal toxicity, was chosen (16, 17). As expected, 0.01% BAC caused an increase in corneal erosion size and lactate concentration, as well as a severe alteration of the corneal structure, as indicated by histology. A breakdown in the corneal barrier function is notable as demonstrated by an increase in fluorescein sodium permeation after BAC application. By contrast, Vismed Multi® application resulted in accelerated corneal healing without signs of toxicity. Here, Vismed Multi® was chosen as negative control, since it is formulated with hyaluronic acid and further ingredients of no known ocular toxicity in the applied dosage (10).

Regarding Cationorm® application, corneal toxicity was determined, which was indicated by a severe corneal epitheliopathy and alterations of the corneal stroma accompanied by metabolic stress. Yet this toxicity is unexpected, because earlier studies reported no toxicity for Cationorm® in cell culture experiments (18). One would expect that this effect is caused by quaternary ammonium toxicity on account of its preservative CKC with its similarities in structure to toxic BAC. In contrast, no toxicity has been shown for CKC when formulated as an emulsion like in Cationorm® and only minor *in vivo*

and ex vivo toxicity has been shown for CKC when applied in phosphate-buffered saline (19, 20). A further aspect is the bioadhesive property of Cationorm®. As a positively charged nanoemulsion, it is believed to bind to negatively charged glycosyl aminoglycans lining the ocular surface (12) and thereby prolonging the residence time of the formulation on the ocular surface. This, on the other hand, could hinder epithelial cell migration onto denuded stromal areas of erosions to heal the lesion. An influencing factor for the determined corneal toxicity could be the relatively low pH value of 5.5 (0.067 M) of Cationorm® or a combination of the aforementioned factors. Interestingly, we have only seen a minor increase in fluorescein sodium permeability for Cationorm® in comparison to a 10 times higher increase for BAC. Since BAC has demonstrated corneal epitheliopathy not much higher than that shown for Cationorm®, the drug itself bound to the ocular surface could interfere with permeation with the negative loaded fluorescein ion and the positive loaded sodium ion.

The toxicity demonstrated here is due to an overdose of Cationorm® where Cationorm® was applied hourly as opposed to the recommended maximum of 5 doses daily. Thus, to yield indication conform results, it would be necessary to apply this substance only 5 times daily on intact cornea as well as previously damaged cornea. Clinically, a phase III, multicenter study where Cationorm was applied in recommended doses has shown superiority of Cationorm® to hyaluronic acid with a reduction of the ocular surface staining score after 1 and 3 months (21).

Nevertheless, overdosing of Cationorm® certainly might result in severe alteration of the corneal structure and metabolism. This effect should be clinically clarified to indicate whether the application frequency of Cationorm® should be limited to a certain daily frequency.

Corneas, to which Optive® was applied, showed accelerated healing, which was already visible on the second day. Only one cornea demonstrated a small reappearance of corneal erosion on the third experiment day. Whether this effect is an artifact or a sign of toxicity is not clear. A toxic effect could be caused by its preservative, Purite®, although it is only classified as a mild eye irritant based on rabbit studies (Environmental Protection Agency Category II) (22). The reason why one cornea demonstrated a higher cytotoxicity is not understood. Nevertheless, in a previous study we found the integrity of corneal epithelium altered by Optive® with fluorescein stippling in all cases (15). A longer follow-up study lasting more than 3 days of culturing should be performed for the sake of clarification.

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Disclosures

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