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Ocular Tolerance of Absorption Enhancers in Ophthalmic Preparations

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ABSTRACT The use of absorption promoters is a way to improve the bioavailability and therapeutic response of topically applied ophthalmic drugs. The ocular tolerance of 9 potential absorption promoters was investigated as well as the influence of the enhancers' concentration on the ocular tolerance. The substances tested were instillated repetitively (4 times per day, during 3 days, and once just before examination) as aqueous solutions onto rabbit corneas. Fluorescein dyeing enabled us to specifically mark corneal damage that was observed by confocal microscopy. The degree of corneal injury was assessed with an image-processing system that calculated the total fluorescent areas. Confocal microscopy results showed the relatively good tolerance of permeation enhancers like dimethyl sulfoxide (DMSO), decamethonium, edetate, glycocholate, and cholate in contrast to the poorly tolerated saponin and fusidate. Increasing the promoters' concentration led generally to an increase in corneal lesions.

KEYWORDS: ocular absorption, permeation enhancers, confocal microscopy, toxicity.

INTRODUCTION

Over the years, numerous attempts have been made to improve ocular topical bioavailability[1] . Among these attempts, the use of permeation/absorption enhancers/promoters has been proposed[2,3] . These latter substances, initially conceived for percutaneous application, have recently been introduced in ophthalmic preparations[4] .

Absorption promoters are chemicals that modify transiently the integrity of the corneal epithelium, thus promoting the penetration of drugs through the cornea[5] .

Most of the investigations performed with absorption enhancers have focused on the modified corneal permeability of drugs like beta-blockers in rabbit eye[4,6] or the systemic delivery of insulin in rabbit or cat eye[7-9] , but little is known about the ocular tolerance of permeation enhancers. Some studies have assessed in vivo the macroscopic changes in the rabbit cornea, conjunctiva, and iris[4,8,10,11] , while others used microscopic systems to evaluate in vitro the ocular damage caused by absorption enhancers[12,13] . Recently, confocal microscopy has proved to be a useful tool for the in vivo investigation of the cornea, especially for noninvasive assessment of cornea lesions[14] .

At present, there are no marketed ophthalmic preparations containing an absorption promoter, because the ocular tolerance of these excipients is poorly investigated. The purpose of this study was to investigate the possible ocular adverse effects of various absorption enhancers. Additionally, the influence of the enhancers' concentration on the ocular tolerance was assessed.

MATERIALS AND METHODS

Chemicals

Dimethyl sulfoxide (DMSO) and sodium edetate USP XXII (Na_2 EDTA) were purchased from Fluka Chemie AG (Buchs, Switzerland). The bile salts (sodium cholate and sodium glycocholate), as well as sodium fusidate, saponin from quillaja bark, and decamethonium bromide were obtained from Sigma Chemical Co (St Louis, MO). Polyoxyethylene sorbitan monolaurate or Polysorbate 20 (Tween 20) was supplied by Fluka Chemie AG (Buchs, Switzerland). Polyoxyethylene (23) lauryl ether (Brij 35) was a gift from ICI (Essen, Germany). Sodium fluorescein was obtained from Reactolab (Servion, Switzerland). All other chemicals used were of

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analytical grade. All the solutions were freshly prepared in bidistilled water. Solutions were adjusted to the isocryoscopicity of tears by addition of sodium chloride. The cryoscopicity of the solutions measured with a vapor pressure osmometer (Wescor 5500, Baumann-Medical, Wetzikon, Switzerland) ranged between 285 and 300 mmol/kg. The solutions were not buffered with pH ranges between 4.5 and 7.4.

Animals

New Zealand albino rabbits of either sex, weighing between 4.0 and 5.0 kg were individua lly housed in an air-conditioned and light-controlled room at 19°C ± 1°C and 50% ± 5% relative humidity. They were given a standard pellet diet and water ad libitum. All animals were healthy and free of clinically observable ocular abnormalities.

All experiments in the present study conformed to the ARVO (Association for Research in Vision and Ophthalmology) resolution on the human use of animals in ophthalmic and vision research[15] and were approved by the local ethics committees for animal experimentation.

Test procedure

The procedure for instilling test solutions and sedating the rabbit was previously described in detail[16] . Briefly, the test solution (25µL) was applied directly onto the rabbit right cornea 4 times per day for 3 days at 2.5-hour intervals and once on the fourth day. The animals were then sedated with an intramuscular injection of ketamine HCl (15 mg/kg body weight) and xylazine (3mg/kg). The injured corneal areas were labeled by instilling a sodium fluorescein solution 0.5% (25µL). After 2 minutes of dyeing, the excess fluorescein was washed out during 1 minute with a NaCl 0.9% solution at 37°C and the cornea was observed under the confocal microscope. Each test was carried out on 3 rabbits. Instillation of a NaCl 0.9% solution was used as control.

Confocal laser scanning microscopy

A confocal laser scanning ophthalmoscope (CLSO Zeiss, Oberkochen, Germany) modified by addition of a set of lenses in order to view the cornea instead of the retina was used. The instrumental setup has been described earlier[17] . An argon ion laser operating at 488-nm wavelength was used as the excitation light source. The fluorescence signal was detected by a photomultiplier. Images were obtained using an Epiplan-Neofluar 5x/0.15 Numerical aperture (NA) objective lens (Zeiss, Oberkochen, Germany). Optical sectioning was performed parallel to the corneal surface, at 16 equidistant different focal planes, the focus shifting (from 0 to 470 µm) to cover the whole corneal thickness. The images were displayed on a digital video monitor and recorded on an S-VHS videotape. An image-processing system (MicroSemper 6, Synoptics Ldt, Cambridge, UK) carried out the following steps: addition of the 16 digitized images in a stack to produce a 3-dimensional reconstruction, projection of this stack, and calculation of the total surface of the fluorescent areas on the projection. Results were evaluated using the Student t test (unpaired samples, level of significance: $P < .05$).

RESULTS

The percentage of corneal surface damage induced by the instillation of absorption enhancers is shown in **Figure 1** . All tested absorption enhancers were assessed for eye tolerance at a concentration of 1%.

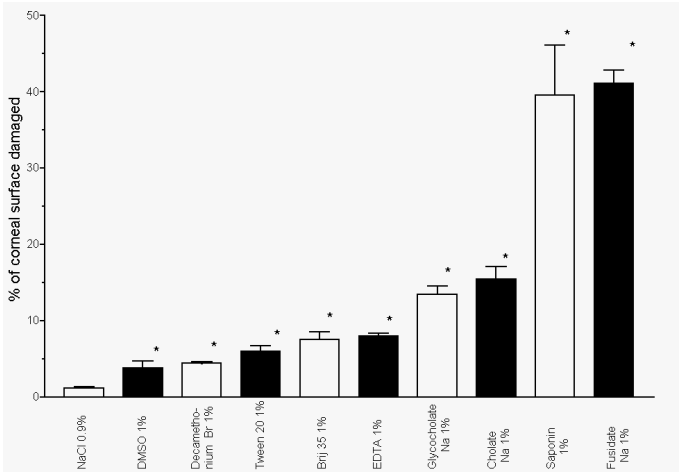

Figure 1. Extent of corneal surface damage produced by instilling absorption enhancers compared to a sodium chloride solution (0.9%). Mean ± SD (n = 3), Student *t* test: **P* < .05.

Compared to the physiological saline solution, all tested absorption enhancers produced a greater fluorescent surface. At an equal concentration of 1%, the rank order for irritancy was DMSO ≅ decamethonium < Tween 20 < Brij 35 ≅ EDTA < glycocholate ≅ cholate << saponin < fusidate. The first 7 substances caused mild irritation: actually less than 16 % of the corneal surface was damaged. With sodium fusidate and saponin, more than 30% of the cornea was injured.

The effect of increasing the concentration of some penetration enhancers on the ocular tolerance is shown in **Figure 2** . All tested enhancers were used at 0.5% and 1% (wt/vol). Doubling the concentration led to various effects according to the nature of the permeation enhancers. The most pronounced effect was seen for sodium cholate: at 1% (wt/vol), the damaged corneal surface was 4fold larger than at 0.5% (wt/vol), whereas doubling the concentration had only a small influence for Tween 20, Brij 35, and saponin.

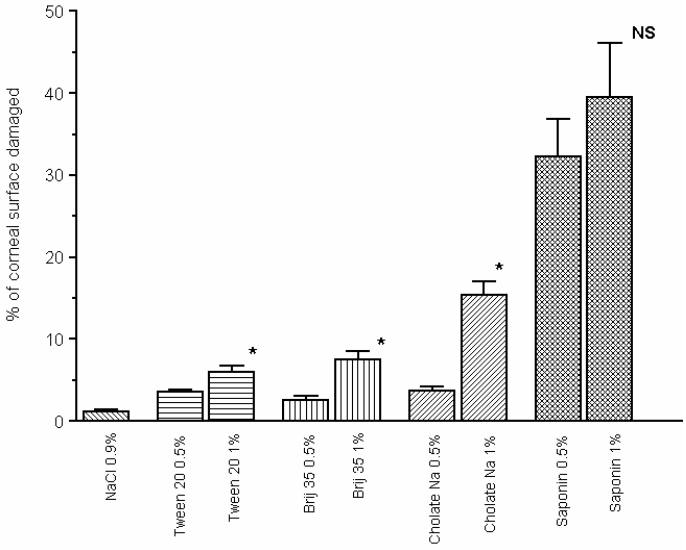

Figure 2. Influence of the instilled concentration of some absorption promoters on the extent of corneal surface damage. Each absorption promoter is tested at 2 concentrations: 0.5% and 1%. The score of the higher concentration is compared to the lower. Mean ± SD (n = 3), Student *t* test: NS = not significant; **P* < .05.

An illustration of the different degrees of corneal damage induced by 3 absorption promoters at 1% (wt/vol)-DMSO, sodium glycocholate, and sodium fusidate-as revealed by the confocal fluorescent images is given in **Figure 3** . Wounded areas are seen as bright spots.

DISCUSSION

Absorption enhancers increase transitorily the permeability characteristics of physiological membranes and are used to facilitate drug penetration through the skin, the cornea and different epithelia (buccal, nasal, intestinal, rectal)[10] . The use of absorption promoters was thought to be helpful in the formulation of ophthalmic preparations to increase therapeutic action of a drug or achieve an equivalent effect with a lower concentration of the active ingredients[1,4,12] .

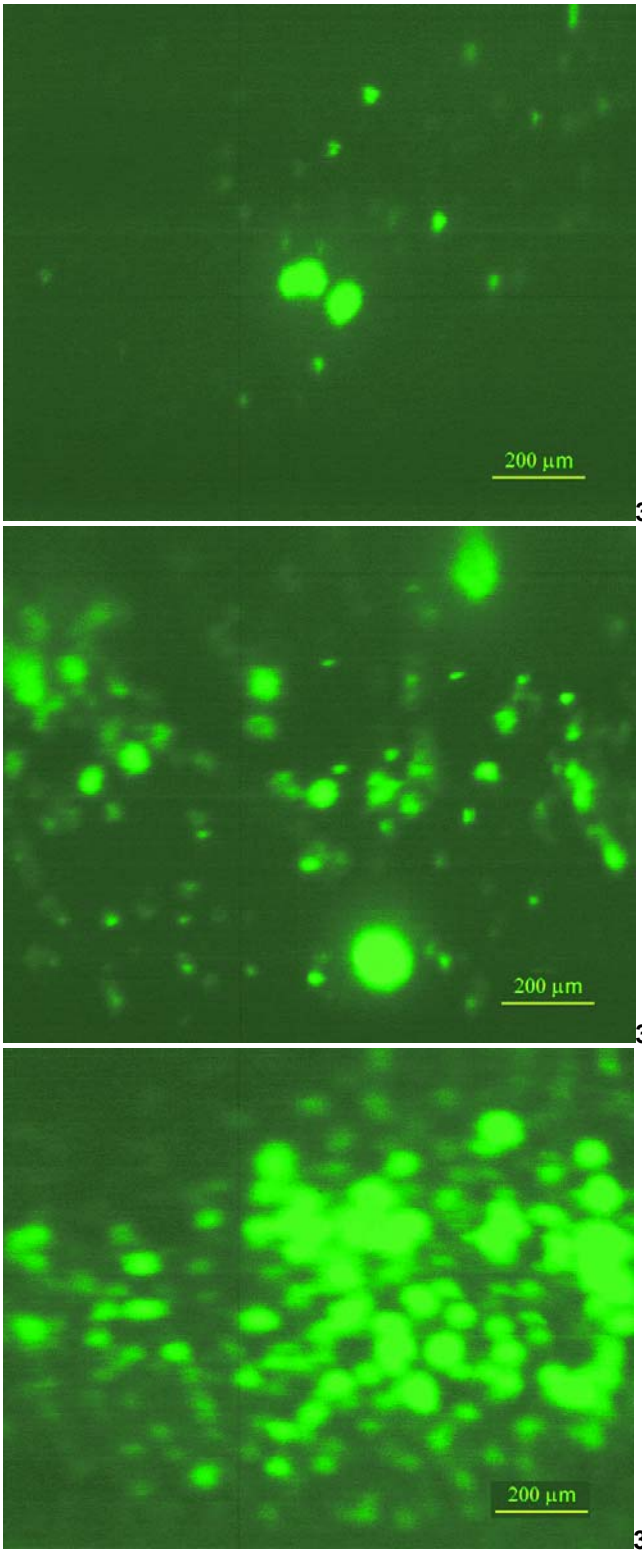

Figure 3. Comparison of the corneal irritation on rabbit corneas produced by the iterative instillation of 3 different absorption enhancers: DMSO (A), sodium glycocholate (B), and fusidate (C), all at 1%. Fluorescent images are the result of stacking 16 optical sections through the whole cornea thickness. The bright areas correspond to damaged zones. The total wounded surface represents 2.5% of the observed field (A); 14.7% in B, and 38.2% in C. The scale bar is 200 µm.

However, artificially increasing the corneal permeability may bring with it the risk of tissue damage[12] . Indeed, despite their effectiveness, most of the penetration enhancers often cause problems associated with ocular irritation and damage[10] .

Our findings show that 2 groups of enhancers can be distinguished according to their irritation potential at 1% concentration: the well tolerated (DMSO, decamethonium, EDTA, glychocolate and cholate, Tween 20, and Brij 35) and the poorly tolerated (saponin and fusidate). Saettone et al[10] , using the Draize irritation test, also reported a good tolerance with EDTA, Brij 78/35, and bile salts (1%) and noticed irritation with saponin (1%). This latter chemical, a natural glycoside with surfactant properties, has been reported to cause irritation and to increase the blinking rate in rabbits[4] . In addition to the ocular tolerance, the enhancing effect is another important issue. An ideal enhancer for ophthalmic preparations should have low irritation and high enhancing effect. Unfortunately, none of the enhancers fulfills both of these requirements at the present time. Actually, a high enhancing effect is certainly associated with ocular damaging effect and, hence, poor tolerance.

With the dosage frequency and the length of exposure, the concentration is an important factor affecting adverse effects of ophthalmic drugs[11] . The efficacy of ocular absorption enhancers has been usually tested at a 0.5% or 1% (wt/vol) concentration[7,18] . Doubling the concentration led in our study to an increase in irritation, but the extent of such an increase depended on the type of promoter. For instance, as shown with our test, doubling the concentration had little effect on the irritation potential in the case of Tween 20, in contrast to sodium cholate. Thus, it can be assumed that the toxicological safety margin of cholate is narrower than the one of Tween 20.

This may be explained by the fact that the chemicals tested have different toxicological dose-response curves. Thus, reducing the concentration by half does not always reduce the toxicity accordingly. Saettone et al[10] also observed that irritation scores do not double with the concentration; for instance, the irritation score for saponin increased from 30 (at 0.5% concentration) to 56 (at 1%), and from 68 to 148 for the bile salt sodium deoxycholate. The in vitro evaluation of the potential cytotoxicity of permeation enhancers confirmed the dose-dependent cytotoxicity of the products[19] .

The appearance of the corneal surface after fluorescent dyeing (**Figure 3**) is consistent with the description made by Norn[20] and Tabery[21] . The typical aspect of fluorescent dots is known as micropunctate fluorescein staining[19] . This staining discloses disruptions of intercellular junctions during the exfoliative process and after corneal lesions resulting from infections, mechanical or toxic injuries, or keratoconjunctivitis sicca[21] .

CONCLUSION

Before choosing an absorption promoter to facilitate ocular absorption of drugs in ophthalmic dosage forms, one must keep in mind the possible adverse effects caused by promoters and assess the benefits and risks of penetration enhancers. An ocular toxicological evaluation of the pharmaceutical preparation containing the permeation enhancer is therefore absolutely necessary. To identify effective permeation enhancers that could be recognized as safe and suitable for clinical use, further in vivo investigations on the effects of associating permeation enhancers with drugs and excipients are needed. Currently studies are in progress to evaluate the wound-healing rate after the use of permeation enhancers.

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