Effect of topical anesthetic agents and ethanol on corneoepithelial wound healing in an ex vivo whole-globe porcine model

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PURPOSE: To assess the impact of topical anesthetic agents and ethanol on ocular surface wound healing using an ex vivo whole-globe porcine model.

SETTING: Department of Ophthalmology, Inselspital, University of Bern, Bern, Switzerland.

DESIGN: Experimental study.

METHODS: Standardized corneoepithelial lesions (5.0 mm diameter, 40 μm depth) were created with excimer laser light in freshly enucleated porcine eyes. The globes (6 per group) were exposed to different concentrations of ethanol (2.0% to 99.0%), cocaine (2.0% to 10.0%), procaine hydrochloride (0.4%), tetracaine (0.5% to 1.0%), or lidocaine (2.0%), 3 drops/hour for 3 hours. Control solutions were physiologic saline, balanced salt solution, and tissue-culture medium. After 20 to 26 hours, wound-healing response was compared by measuring the diameter of each corneoepithelial lesion.

RESULTS: The mean diameter of corneoepithelial lesions exposed to physiologic saline decreased from 4.78 mm $\pm$ 0.19 (SD) to 4.44 $\pm$ 0.17 mm between 20 and 26 hours. After 24 hours, the mean lesion size, compared with physiological saline, was larger after cocaine 5.0% (5.20 $\pm$ 0.26 mm) and 10.0% (5.39 $\pm$ 0.12 mm), tetracaine 0.5% (5.59 $\pm$ 0.35 mm) and 1.0% (5.55 $\pm$ 0.27 mm), and procaine hydrochloride 0.4% (5.76 $\pm$ 0.12 mm), but not after lidocaine 2.0% (5.01 $\pm$ 0.17 mm). Balanced salt solution, tissue-culture medium, ethanol 2.0% to 99.0%, and cocaine 2.0% did not inhibit the wound-healing response.

CONCLUSIONS: In an ex vivo whole-globe porcine model, lidocaine 2.0% and cocaine 2.0% were the least toxic anesthetic agents. At all concentrations, ethanol had no impact on wound healing.

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Wound healing of the ocular surface is crucial for its functional outcome after damage to the corneal epithelium. Such damage can be induced not only by mechanical injury but also by contact lens wear and by the topical application of anesthetic agents or ethanol.[1–3] Topical anesthetic agents are used in anterior segment surgery[4,5] and for postoperative analgesia after photorefractive keratectomy (PRK),[6,7] although their toxicity is under debate.[6,8–10] Ethanol is used to create epithelial flaps in laser light–induced epithelial keratomileusis.[11–13] The aim of the present study was to assess the impact of topically applied anesthetic agents and ethanol on the wound-healing response of the ocular surface in an ex vivo whole-globe porcine model.

MATERIALS AND METHODS

Experimental Setup

The eyes of freshly slaughtered pigs were obtained within 3 hours of death from a local abattoir. Only eyes with a macroscopically clear cornea and an intact epithelium were used.

Standardized corneoepithelial lesions, 5.0 mm in diameter and 40 μm in depth, were created using an excimer laser (Schwind Eye-Tech-Solutions GmbH & Co. KG) with a wavelength of 193 nm and a light frequency of 13 Hz according to a standard protocol for PRK.[14] In contrast to an earlier

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investigation in which the whole-globe ex vivo porcine model was first described,[15] a quantitative assessment of the repair response was imperative for the present study. In the earlier investigation, lesions with an ablation diameter of 1.5 to 2.0 mm were created. Because such small lesions heal completely within 24 to 28 hours, they are not suited for quantitative evaluation of this process. As a consequence, the diameter of the ablation zone was increased to 5.0 mm. Furthermore, the depth of the ablation zone was decreased from 70 μm to 40 μm to avoid disturbances in the corneal stroma, which could interfere with the speed of reepithelialization. In addition, the incubation time was reduced from 40 to 25 hours to avert a possible impact of postmortem tissue changes.

After laser treatment, the eyes were mounted on a purpose-built support that permitted their upright positioning. To maintain a physiologic intraocular pressure, the vitreal cavity was first cannulated with a 25-gauge needle via the pars plana and then infused with tissue-culture medium containing a 1.0% antibiotic/antimycotic solution (Invitrogen Corp.) from 20 cm above the ocular surface (Figure 1, *A*).

Topical Application of Control and Test Solutions

Three drops of tissue-culture medium (Hospital Pharmacy, Inselspital, Bern, Switzerland) were applied to the ocular surface of each eye, which was then allowed to equilibrate for 1 hour at 36°C in a humidified atmosphere before the control or test solution was applied: Preservative-free solutions of ethanol (2.0%, 5.0%, 10.0%, 20.0%, 50.0%, and 99.0%, diluted with a balanced salt solution), cocaine (2.0%, 5.0%, and 10.0%; Hospital Pharmacy Inselspital), tetracaine (0.5%, and 1.0%), lidocaine hydrochloride 2.0%, or procaine hydrochloride 0.4% (Novocain) containing chlorhexidine acetate as a preservative (Novesin). Control solutions were physiologic saline (0.9%), balanced salt solution (pH 7.4), and tissue-culture medium. Each eye received 3 drops per hour for 3 hours. The ocular surface was then moistened with tissue-culture medium at a rate of 3 drops per hour for an additional 2 hours. Thereafter, the eyes were incubated for a further 14 hours at 36°C in a humidified atmosphere.

The pH values of the tissue-culture medium and the balanced salt solution correspond to the slight alkalinity of the normal human tear film (pH 7.3 to 7.7), whereas the anesthetic solutions are acidic (cocaine hydrochloride 10.0%: pH 3.8; cocaine hydrochloride 5.0%: pH 6.2; cocaine hydrochloride 2.0%: pH 3.8; lidocaine 2.0%: pH 5.9; procaine hydrochloride 0.4%: pH 4.8; tetracaine 1.0%: pH 5.1; tetracaine 0.5%: pH 6.4). The osmolarities of physiological saline (282 mOsm), balanced salt solution (244 mOsm), tissue-culture medium (253 mOsm), cocaine hydrochloride 2.0% (299 mOsm), lidocaine 2.0% (300 mOsm), and procaine hydrochloride 0.4% (287 mOsm) lay within the range that is characteristic of the healthy human tear film (244 to 344 mOsm). The osmolarities of 5.0% and 10.0% cocaine are higher (377 and 441 mOsm, respectively), whereas those of 0.5% and 1.0% tetracaine lay below the detection limit. Depending on the dilution factor, solutions of ethanol were hypotonic (eg, ethanol 2.0%) or highly hypertonic (eg, ethanol 99.0%).

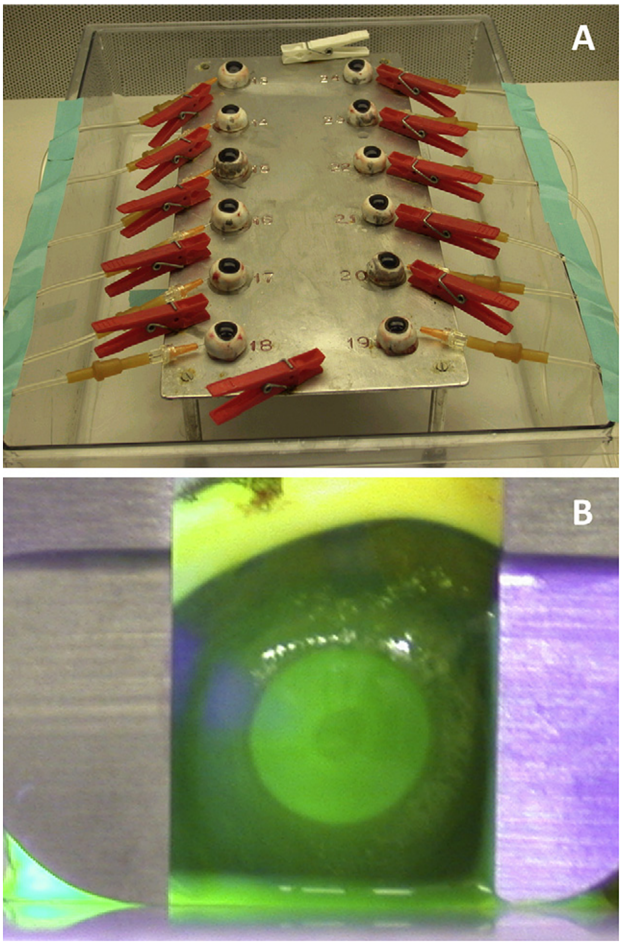

Figure 1. Standardized corneoepithelial lesions were created with an excimer laser. *A*: Vitreous cavity was cannulated with a 25-gauge needle through the pars plana and then infused with tissue-culture medium. *B*: Example of a corneoepithelial lesion after staining with fluorescein and ultraviolet-light illumination.

Lesion Size Measurement

The diameter of each corneoepithelial lesion was determined 20, 22, 24, and 26 hours after its creation, following an initial washing of the ocular surface with tissue-culture medium, its staining with 2 drops of fluorescein 0.5% (Alcon Laboratories, Inc.), and a final rinsing with tissue-culture medium (Figure 1, *B*). Under conditions of ultraviolet light

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illumination, the horizontal and vertical diameters of each lesion were measured with a gauge. The mean lesion diameter and the standard deviation (SD) were calculated for each group (n = 6).

Assumptions

Inhibition of the wound-healing response was assumed to have occurred if the diameter of the lesion was larger after exposure to a test solution than to physiologic saline. The test solution was deemed to have had a toxic influence if the diameter of the lesion exceeded 5.0 mm (the initial value) 20 hours after its creation.

Statistical Analysis

Statistical analysis was performed using GraphPad software (version 5.0c, GraphPad Software, Inc). Mean values (±SD) were calculated. Intergroup comparisons were based on a 1-way analysis of variance and the Dunnett multiple comparison post hoc test. The level for statistical significance was set at a P value of 0.05.

RESULTS

Figure 2 shows the mean diameters of the corneoepithelial lesions at the 24-hour juncture. After the application of physiologic saline 0.9%, the mean diameter of the lesions was 4.78 mm ± 0.19 (SD) after 20 hours, 4.63 ± 0.20 mm after 22 hours, 4.53 ± 0.21 mm after 24 hours, and 4.44 ± 0.17 mm after 26 hours, corresponding to a mean reepithelialization speed of 0.056 ± 0.016 mm per hour. Lesions that had been exposed to balanced salt solution or tissue-culture medium had comparatively smaller diameters (4.38 ± 0.09 mm and 4.28 ± 0.08 mm, respectively), at the 26-hour juncture, although the differences were not statistically significant ($P > .05$) (Figure 3, *A*).

Irrespective of concentration, 2.0% to 99.0% ethanol solutions that had been prepared using balanced salt solution as a diluent had no significant influence on the wound-healing response ($P > .05$) (Figure 3, *B*). At a concentration of 2.0%, cocaine had no adverse effect on wound healing; however, at higher concentrations (5.0% and 10.0%), it inhibited the repair response at each monitoring time ($P < .001$) (Figure 3, *C*). Tetracaine 0.5% or 1.0% and procaine hydrochloride 0.4% also inhibited corneal reepithelialization at all measured time points ($P < .001$) (Figure 3, *D*). Lidocaine 2.0% had no significant influence on wound healing ($P > .05$).

DISCUSSION

The data in our study using an ex vivo whole-globe porcine model of corneoepithelial wound healing confirm on a quantitative basis previous clinical findings relating to the toxicity of the tested topical anesthetic agents.[9,16–18] At concentrations higher than 2.0%, cocaine suppressed reepithelialization of the cornea in a dose-dependent manner during the entire monitoring period, whereas ethanol had no such inhibitory effect. Dilute alcohol has now been used for more than a decade in the context of photorefractive surgery.[11,12] In laser-assisted subepithelial keratectomy, a 20.0% ethanol solution is applied for 20 to 30 seconds via an optical funnel to the ocular surface to loosen the epithelium from Bowman membrane without causing cellular or cohesion damage.[19,20]

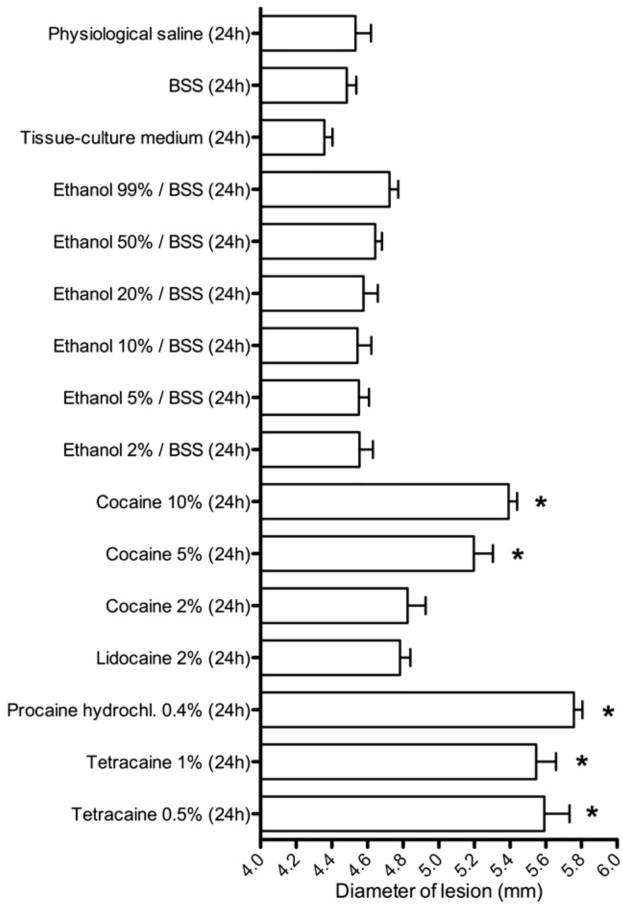

Figure 2. Diameters of corneoepithelial lesions 24 hours after exposing the ocular surface of ex vivo porcine globes to the indicated solutions (* = $P < .001$ compared with physiologic 0.9% saline, which served as the basis for the intergroup statistical comparison). Mean values (6 eyes per group) are represented with the SDs (*bars*) (BSS = balanced salt solution).

With the exception of lidocaine 2.0%, each of the other tested topical anesthetic agents proved to be remarkably toxic to the ocular surface at clinically relevant concentrations. Given this finding, it is astonishing that problems with the ocular surface have not been more frequently encountered with their use. In our model, the anesthetic agents were applied 3 times per hour over 3 hours, whereas a single application would be deemed sufficient in a clinical setting. Nevertheless, the self-administration of topical anesthetic agents can be abused by frequent applications (eg, to relieve corneal pain), and toxicity may become a relevant issue.[3,21]

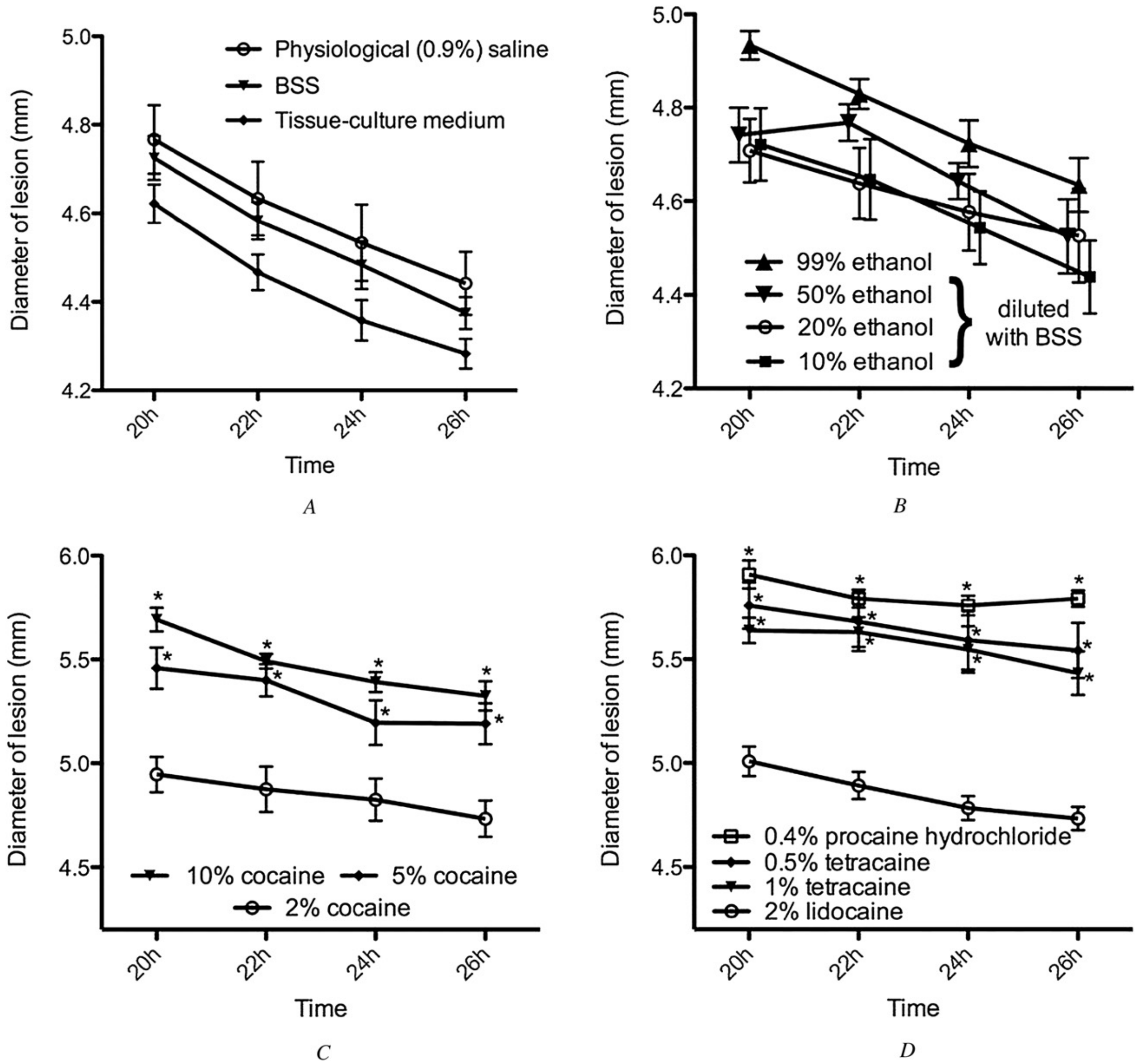

Figure 3. Temporal course of corneoepithelial wound healing after exposing the ocular surface of ex vivo porcine globes to physiologic 0.9% saline (*A*), balanced salt solution (*A*), tissue-culture medium (*A*), ethanol (*B*), cocaine (*C*), procaine hydrochloride (*D*), tetracaine (*D*), or lidocaine (*D*). Mean values (6 eyes per group) are represented with the SDs (*bars*). The mean lesion diameter in eyes that had been exposed to physiologic saline 0.9% served as the basis for the intergroup statistical comparison at the different time points (* = $P < .001$; BSS = balanced salt solution).

Of the tested topical anesthetic agents, procaine hydrochloride, which is more frequently used than any other topical anesthetic in ocular surgery, was the most toxic. The presence of chlorhexidine as a preservative in commercially available preparations of procaine hydrochloride may contribute to its inhibitory effect. At least for ocular surface interventions,[22] the use of lidocaine may thus be preferable to that of procaine hydrochloride.

Hyperosmolaric preparations have been shown to induce apoptosis of human corneal epithelial cells.[23] Hyperosmolaric stress can also lead to the dehydration of corneal epithelial cells and thus to an increase in the rate of their desquamation.[24] On the other hand, hypotonic preparations may affect corneal integrity and wound healing.[25,26] All these factors may have modified the toxicity profile of drugs applied to the ocular surface in our wound-healing model as do the same in a clinical setting.

Clearly, studies with porcine tissue may not directly be translated into the human situation. Anatomically and physiologically, however, the corneal epithelia of human and porcine eyes are similar, except that Bowman membrane is thinner or even absent in pigs.[27] As a consequence, porcine eye models are widely used to assess the wound-healing response to penetrating keratoplasty and mechanical or PRK[28–31] since many of the cellular phenomena that comprise the latter process are comparable.[32–34] Hence, the porcine eye is not an inappropriate choice of model to assess the impact of surgical technique and drug toxicity on corneal wound healing.[14,15,29,35,36] In our hands, the ex vivo whole-globe porcine model of corneoepithelial wound healing correlates well with clinical experience[14] and thus limits the need for experiments with living animals. Moreover, the setup permits a distinction between an inhibition of wound healing, which is indicated by a more tardy reduction in lesion size, and drug-induced toxicity per se, which is evinced by an enlargement of the primary lesion.[14]

In conclusion, with the exceptions of cocaine 2.0% and lidocaine 2.0%, the topical anesthetic agents proved to be remarkably toxic at the tested concentrations (cocaine at 5.0% and 10.0%, procaine hydrochloride at 0.4%, and tetracaine at 0.5% and 1.0%). Ethanol, on the other hand, was well tolerated at all tested concentrations (2.0% to 99.0%).

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