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An *Ex-Vivo*, Whole-Globe Porcine Model of Corneoepithelial Wound Healing Tested Using Immunomodulatory Drugs

FRANZISKA FLUECKIGER,[1] LAURENT KODJIKIAN,[2] MARKUS HALBERSTADT,[1] MATTHIAS BOEHNKE,[3] and JUSTUS G. GARWEG[1]

ABSTRACT

Background: **An efficient epithelial wound healing is essential for the preservation of vision. Hence, the effects of novel topical drugs on the ocular surface must be ascertained before clinical use. We have tested the utility of an *ex vivo*, whole-globe porcine screening model to serve as a partial substitute for resource- and time-consuming animal experiments.**

Methods: **Standardized corneoepithelial lesions, 5.0 mm in diameter and 40 μm in depth, were created with an Excimer laser in freshly enucleated porcine eyes. These were then exposed to control solutions (physiological saline (baseline), tissue-culture medium (positive control) and NH_4^+ (toxicity control)) and to three test agents (cyclosporin A, dexamethasone, and mitomycin C). The wound-healing response and toxic effects were monitored after 20–26 h by comparing lesion sizes.**

Results: **According to baseline data obtained using physiological saline, tissue-culture medium improved wound healing. The highest doses of NH_4^+ (1 M) and mitomycin C (1.0 mg/mL) elicited toxic effects (confidence interval according to Scheffé's post hoc test: −0.65 to −0.07 and −0.99 to −0.60, respectively). Under the same test conditions, cyclosporin A (0.1 to 10 mg/mL) and dexamethasone (0.1 to 10 mg/mL) had no influence on corneoepithelial wound healing.**

Conclusions: **Drug screening with this *ex vivo* porcine model permits a reproducibly quantitative and time- and dose-dependent assessment of corneoepithelial wound healing. This model corresponds more closely to the clinical situation than cell culturing and may, therefore, be useful in evaluating novel pharmaceutical agents, thereby helping to cut down on the number of animal experiments performed prior to the instigation of clinical trials.**

INTRODUCTION

FOLLOWING OCULAR SURGERY OR TRAUMA, a rapid and complete repair of the corneal epithelium, which involves its renewal, is essential for the maintenance of corneal transparency and normal visual acuity.[1,2] During the past few years, many diseases have been recognized to interfere with the stability of the ocular surface. Hence, the search for new drugs that stabilize the corneal epithelium has been, and still is, intensive.[3–7] Before being approved by federal law for clinical

[1]Department of Ophthalmology, Inselspital, University of Bern, Bern, Switzerland.
[2]Service d'ophtalmologie, CHU de la Croix-Rousse, Claude Bernard Lyon I University, Lyon, France.
[3]Institute for Ophthalmology, Hamburg, Germany.

use in humans, candidate drugs are subjected to a series of tests to assess both their impact on wound healing and their toxicity.

The standard procedure is a three-stage process, which involves cell-culturing experiments to evaluate the rate of proliferation and the metabolism of the corneal epithelium, testing in living animals to assess wound healing (i.e., re-epithelialization) and, finally, preclinical human trials. The initial two phases are time- and resource-consuming, and a simplification of the testing procedure instigated prior to the onset of human clinical trials would be greatly valued.

In rabbits, wound healing of a traumatized corneal epithelium is a biphasic process, irrespective of the lesion size, with an initial latent phase being followed by a linear epithelial healing phase at a rate of 64 ($\pm$ 2) μm/h.[1] Such a linear wound-healing process would serve as an excellent basis for assessing the temporal course of corneoepithelial wound healing in response to locally applied pharmaceutical agents. If an appropriate *ex vivo* model of this linear corneoepithelial wound-healing process could be established, we would be furnished with a simple means of accurately assessing the kinetics and therapeutic modulation of the response in a system that simulated the *in vivo* situation more closely than a cell-culturing one. With this end in view, we set up an *ex vivo* model using freshly enucleated porcine eyes. To test the model, we first applied physiological saline (baseline control) to establish the baseline level of corneoepithelial wound healing. A nutrient-rich tissue-culture medium was applied as a positive control and an alkaline agent (NH_4^+) as a toxicity control. Three drugs that are used topically in ophthalmology to modify wound healing (i.e., cyclosporin A, dexamethasone, and mitomycin C) were then tested at different concentrations. Our aim was to establish a system that could be used to reliably predict the outcome of animal experiments, thereby permitting these to be reduced to a minimum.

METHODS

Materials

Source of tissue for ex-vivo *experiments*

The eyes of freshly slaughtered pigs were obtained within 3 h of death from a local abattoir. Only those with a macroscopically intact corneal surface were further processed. Porcine eyes are anatomically similar to human eyes[8,9] and can be obtained almost worldwide—not only in large numbers but also in an excellent and reproducible biological condition. In this study, the number of eyes utilized in parallel experiments was limited to 24 in order to avoid technical artefacts, such as drying of the ocular surface. Hence, six eyes were used in each of four parallel experiments to evaluate the putative effect of each tested substance and concentration. One of the four parallel experiments was always represented by a baseline control group of eyes (treated with physiological saline (n = 6)), and each of the other three parallel experiments by one of the three tested concentrations of the applied drug (n = 6 for each concentration).

Controls and test substances

In all wound-healing experiments, physiological (0.9%) saline (Bichsel; Interlaken, Switzerland) served as a baseline (inert) control and tissue-culture medium (minimal essential medium without phenol red [Hospital Pharmacy; Inselspital Bern, Switzerland]), as a positive one. NH_4^+ (Merck; Stettlen, Switzerland) served as a toxicity control for the inhibition of cell proliferation.

Three test substances were evaluated: cyclosporin A (0.1 mg/mL, 1 mg/mL, and 10 mg/mL [Fluka; Buchs, Switzerland]), which was dissolved in ethanol and further diluted in distilled water; dexamethasone (0.1 mg/mL, 1 mg/mL, and 10 mg/mL [Sigma; Buchs, Switzerland]), which was dissolved and further diluted in distilled water; and mitomycin C (0.01 mg/mL, 0.1 mg/mL, and 1 mg/mL [Kyowa, Tokyo, Japan]), which was likewise dissolved and diluted in distilled water.

Preparation of porcine eyes

The eyes were gently rinsed first with water, to remove adhering blood, and then with basic salt solution ((BSS) pH 7.4 (Hospital Pharmacy; Inselspital Bern, Switzerland)). Only eyes that manifested a completely transparent cornea and that evinced no signs of injury in an Excimer-laser operating microscope were included in the experiments. After completely removing the adnexa to minimize the risk of bacterial contamination, the globes were disinfected in 3% povidone-iodine for 1 min, rinsed thoroughly with

BSS, immersed in tissue-culture medium containing 0.1% gentamycin (Essex Chemie; Luzern, Switzerland) for 3 min, again rinsed with BSS, and then placed upon wet swabs within sterile containers to prevent drying of the ocular surface. The entire procedure was conducted under semisterile (laminar-flow) conditions.

Creation of epithelial lesions

Using an Excimer laser (193 nm, 13 Hz; Schwind, Kleinostheim, Germany), a standard photorefractive, keratectomy-like lesion, 5.0 mm in diameter and 40 μm in depth, was created in the corneal epithelium. In preliminary experiments, this size and depth had been established to be optimal for the quantitative assessment of wound healing with time (unpublished data; see also[10]). However, since the postirradiation diameter of the lesion does not always correspond to the calculated value, this parameter was measured immediately after Excimer-laser treatment, as well as at the end of the wound-healing monitoring phase. The lesion diameter measured at the end of the evaluation period was always subtracted from the actual (not from the predicted) postirradiation value.

Experimental setup

After laser treatment, the eyes were mounted on a custom-built support, which permits their upright positioning. To maintain a physiological intraocular pressure, the vitreous cavity was first cannulated with a 25-gauge needle through the pars plana, and then infused with tissue-culture medium containing a 1% antibiotic/antimycotic solution (Invitrogen; Basel, Switzerland) from a height of 20 cm above the ocular surface. The experiments were conducted in a humidified incubator maintained at 36°C.

Application of control and test agents

The ocular surface was moistened by applying three drops of tissue-culture medium within 10 min and permitted to equilibrate in the incubator for 1 h. The test agent or the solvent alone was applied at a rate of three drops per hour (i.e., one drop every 20 min) for 3 h. The ocular surface was then moistened with tissue-culture medium at a rate of three drops per h for an additional 2 h. Thereafter, the eyes were left untreated in the humidified atmosphere of the incubator for a further 14 h (overnight).

Measurement of the lesion size

The experiment was terminated by rinsing the ocular surface first with tissue-culture medium, then with two drops of BSS containing 0.5% fluorescein (Alcon; Hünenberg, Switzerland), and, finally, once again with tissue-culture medium. The size of each lesion was measured horizontally and vertically in two different positions under an ultraviolet light source using a technical gauge with a precision of 10 μm; the results were averaged. This procedure was repeated every 2 h from 20 h to 26 h after the onset of the experiment (i.e., after 20, 22, 24, and 26 h).

Definitions

Corneoepithelial wound healing was defined as a time-dependent reduction in the size of the corneoepithelial lesion relative to the baseline value (5 mm) under physiological conditions (36°C, humidified atmosphere).

Baseline wound healing was defined as the wound healing observed after treatment of the ocular surface with physiological (0.9%) saline without nutrients.

Maximal wound healing was expected after exposing the corneoepithelial lesion to tissue-culture medium.

Inhibition of corneoepithelial wound healing was deemed to have occurred if the size of the lesion did not regress with time relative to the baseline control value (physiological saline).

Ocular surface toxicity was defined as an increase in lesion size above the initial value at 20 h.

Control for ocular surface toxicity: NH_4^+ (1.0 M) was included as a toxicity control (alkaline conditions).

Statistics

The statistical analysis was performed using SPSS for Windows, version 11.5 (SPSS, Chicago, IL). The effect of different concentrations of a tested substance on epithelial wound healing was determined by a univariate analysis of variance amongst repeated measurements. Intergroup comparisons were based on a one-way analysis of variance (ANOVA) (relative to the baseline (physiological saline) and maximum (tissue-culture medium) levels). Scheffé's post hoc test was applied. Data were confirmed to be normally distributed using Shapiro-Wilk statistics. The Lev-

ene test was implemented to establish that the variance was homogeneous. Differences between sets of data were considered to be significant if P values were less than or equal to 0.05 (on the basis of two-tailed tests).

RESULTS

In baseline controls (exposed to *physiological saline*), corneoepithelial lesions that had a mean diameter of 5 mm at the onset of the experiment had diameters of 4.79 (± 0.16) mm and 4.57 (± 0.16) mm after 20 h and 26 h, respectively (Fig. 1). Repeated testing on different occasions yielded similar results for all control experiments (Table 1).

Exposure of the ocular surface to *tissue-culture medium* (positive control) enhanced the wound-healing response after 24 h. The lesion diameter was reduced to 4.24 (± 0.17) mm and 4.18 (± 0.11) mm after 24 and 26 h, respectively (unifactorial ANOVA: $P = 0.008$ and $P = 0.001$, respectively; Table 1).

Exposure of the ocular surface to the highest concentration of NH_4^+ (1.0 M (toxicity control)) had the expected toxic effect. By 20 h, the lesion diameter had increased to 5.3 (± 0.25) mm; $P < 0.01$); but thereafter, wound healing followed a normal course (Table 1; Figure 2A). The lower concentrations of NH_4^+ (0.01 and 0.1 M) had no toxic or inhibitory effect on corneoepithelial wound healing. A comparison of the effects achieved at each concentration using Scheffé's post hoc test confirmed a significant negative impact of the highest concentration of NH_4^+ (1 M) on wound healing (Confidence Interval: −0.65 to −0.07; $P = 0.01$).

All tested controls and test drugs showed, in the univariate analysis of variance, a highly significant dose-dependant impact of the factor time (for each tested agent and concentration: $P < 0.001$).

At concentrations of 0.1 mg/mL and 1.0 mg/mL, *cyclosporin A* had no influence on wound healing ($P > 0.05$). But at 10 mg/mL, it tended to have an inhibitory effect on this response at 22 hours (Table 1, Figure 2B). However, although Scheffé's posthoc test revealed an asymmetric 95% Confidence Interval, the wound-healing response did not differ significantly from the baseline control (physiological saline) (Confidence Interval: −0.102352 to 0.769019; $P = 0.247$).

According to the univariate analysis of variance, *dexamethasone* did not influence corneoepithelial wound healing at any of the tested concentrations (0.1 mg/mL, 1.0 mg/mL), and 10 mg/mL (Table 1, Figure 2C). This result was confirmed after applying Scheffé's posthoc test.

At concentrations of 0.01 and 0.1 mg/mL, *mitomycin C* had no influence on corneoepithelial wound healing according to Scheffé's posthoc test. But at 1.0 mg/mL, it had not only an inhibitory effect on corneoepithelial wound healing but also a toxic one, which was manifested as an increase in the size of the lesion at 20 h. Scheffé's

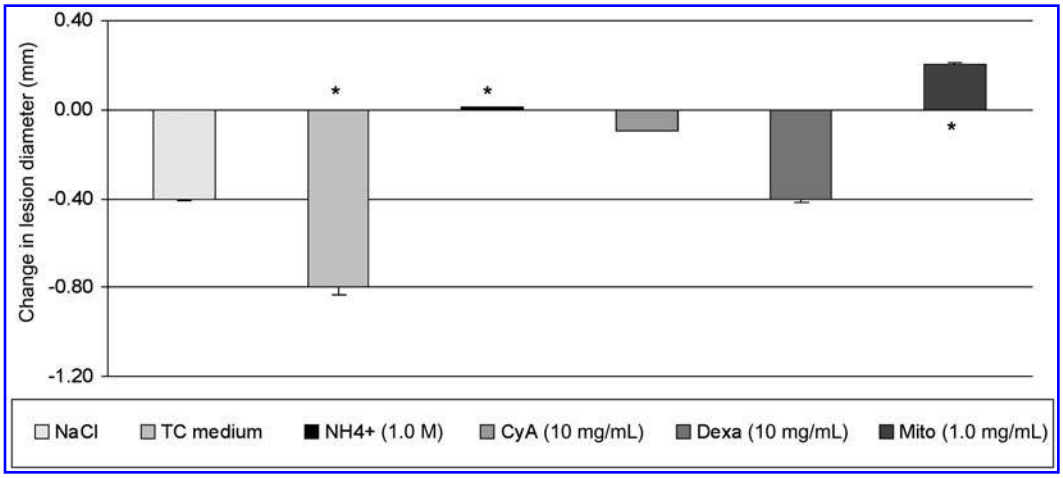

FIG. 1. Change in the defect diameter after 24 h. *Corresponds to a P value 0.05 (Sheffé's posthoc test). NaCl, baseline; TC medium, positive control; $NH4^+$, toxicity control; CyA, cyclosporin A; Dexa, dexamethasone; Mito, mitomycin C.

TABLE 1. LESION DIAMETERS 20 TO 26 HOURS AFTER THE APPLICATION OF TEST SUBSTANCES TO THE OCULAR SURFACE OF *EX VIVO* PORCINE EYES

	20 h			*22 h*			*24 h*			*26 h*		
Time	*Mean*	*SD*	P	*Mean*	*SD*	P	*Mean*	*SD*	P	*Mean*	*SD*	P
Baseline												
NaCl (0.9%)	4.8	0.16		4.5	0.19		4.5	0.13		4.6	0.16	
Toxicity Control												
NH_4^+: 0.01 M	4.9	0.29		4.7	0.16		4.6	0.21		4.6	0.15	
NH_4^+: 0.1 M	4.8	0.26		4.6	0.22		4.6	0.2		4.6	0.18	
NH_4^+: 1.0 M	5.3	0.25	< 0.01	5.1	0.15	< 0.01	5.0	0.17	< 0.01	5.0	0.17	< 0.01
Positive Control												
TC medium	4.8	0.16		4.4	0.15	n.s.	4.2	0.17	< 0.01	4.2	0.11	< 0.01
NaCl (0.9%)	4.8	0.16		4.5	0.19		4.5	0.13		4.6	0.17	
CyA: 0.1 mg/mL	4.8	0.20		4.6	0.26		4.6	0.19		4.6	0.19	
CyA: 1.0 mg/mL	4.6	0.26		4.6	0.24		4.7	0.26		4.8	0.38	
CyA: 10 mg/mL	4.9	0.17	n.s.	5.0	0.24	n.s.	4.9	0.12	n.s.	5.0	0.21	n.s.
NaCl (0.9%)	4.6	0.24		4.5	0.27		4.5	0.28		4.4	0.33	
Dexa: 0.1 mg/mL	4.7	0.23		4.5	0.19		4.5	0.19		4.4	0.17	
Dexa: 1.0 mg/mL	4.7	0.10		4.6	0.14		4.5	0.18		4.4	0.23	
Dexa: 10 mg/mL	4.6	0.22	n.s.	4.6	0.19	n.s.	4.6	0.20	n.s.	4.5	0.16	n.s.
NaCl (0.9%)	4.7	0.12		4.6	0.08		4.6	0.11		4.6	0.08	
Mito: 0.01 mg/mL	4.7	0.13		4.5	0.18		4.4	0.09		4.3	0.12	
Mito: 0.1 mg/mL	5.0	0.16		4.9	0.11		4.6	0.08		4.6	0.09	
Mito: 1.0 mg/mL	5.7	0.23	< 0.001	5.6	0.20	< 0.001	5.2	0.16	< 0.001	5.2	0.17	< 0.001

Mean values (in mm) are presented together with the standard deviation (SD) and *P* values. The *P* values were calculated by comparing the displayed lesion sizes with the baseline value (i.e., 50 mm) using Scheffé's posthoc test. In all groups, the factor time was highly significant (ANOVA: $P < 0.001$).

NH_4^+, ammonium; CyA, cyclosporin A; Dexa, dexamethasone; Mito, mitomycin C; NaCl (0.9%), physiological saline; TC medium, tissue-culture medium; n.s., not significant.

post hoc test revealed the inhibitory effect to be significant (relative to the base-line control (physiological saline)) over the entire monitoring period (Confidence Interval: −0.992367 to −0.603466; $P < 0.001$; Table 1, Figure 2D).

DISCUSSION

We have established an *ex vivo* photorefractive, keratectomy-like screening model for epithelial wound healing of the ocular surface in porcine eyes. Our model enabled us to quantify this parameter with time and with increasing drug dose, thereby permitting a distinction between the inhibition of wound healing and the evocation of toxic effects. It may be of use in gathering toxicity information and could serve as a partial substitute for the expensive and time-consuming experiments in living animals that precede the clinical testing of a drug. As yet, the quality of wound healing has not been addressed. But this parameter could be readily assessed in the future by monitoring, for example, lactate dehydrogenase activity.[11]

In contrast to most of the established corneoepithelial wound-healing models, in which the defects are generated chemically or mechanically, ablation with the Excimer laser creates lesions of a reproducibly precise depth and diameter.[10,12,13] Because the standard error of the mean is thereby lowered, the number of experiments that need to be conducted can be reduced. Moreover, the penetration depth of Excimer-laser radiation ($\lambda = 193$ nm) is extremely shallow, and the collateral damage to adjacent tissues is, consequently, much less than when lesions are generated chemically or mechanically.[14] This circumstance will also improve the reproducibility of the results.

Another advantage of our *ex vivo* model is that it does not depend upon the use of living animals. Hence, it is subject neither to the legal restrictions associated with animal experiments nor to the steep costs of maintaining laboratory animals, according to GLP guidelines and their ultimate sacrifice. Furthermore, when using living animals, the ocular irritation caused by a locally applied drug will induce a wiping of the affected eye, which could interfere with an accurate determi-

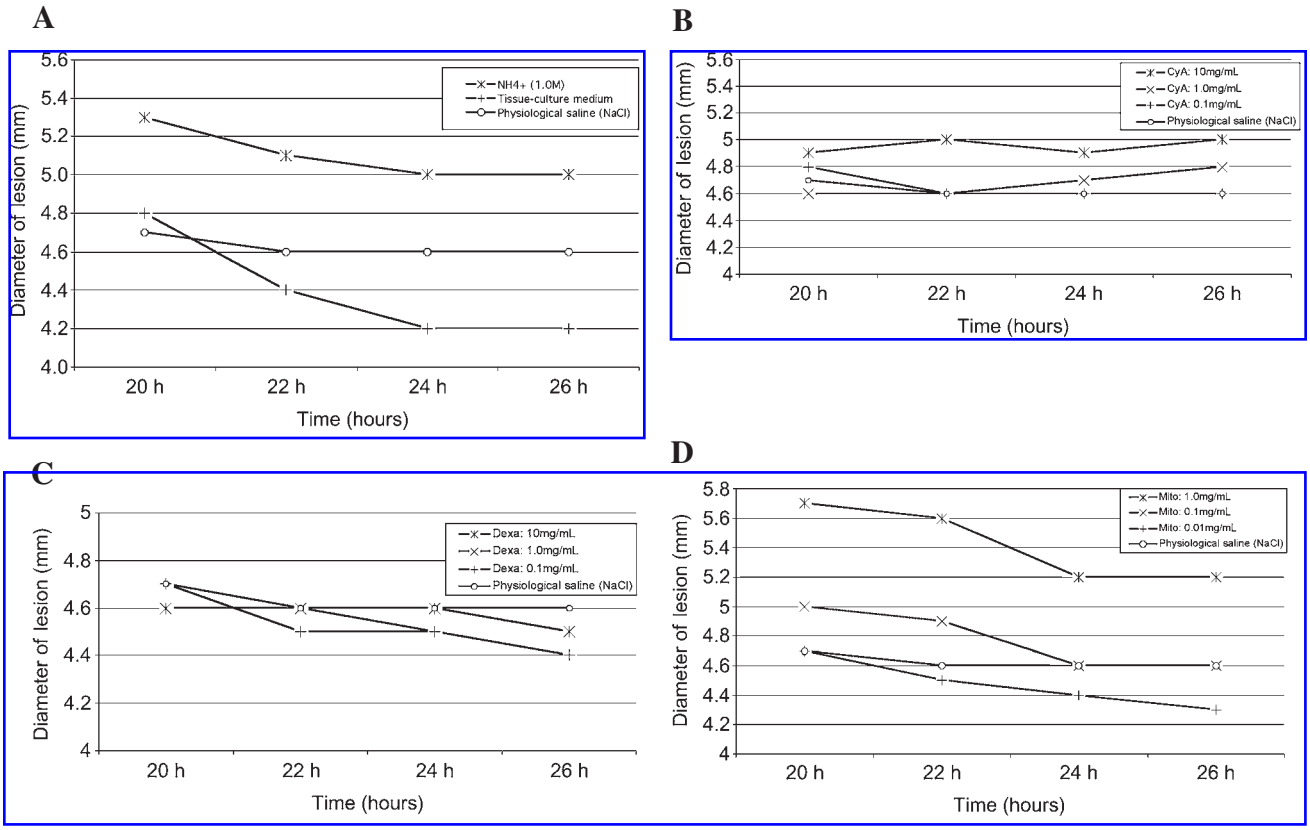

FIG. 2. (**A**) Temporal course of wound healing after exposure of the ocular surface to different control solutions. (**B**) Temporal course of wound healing after exposure of the ocular surface to cyclosporin A. (**C**) Temporal course of wound healing after exposure of the ocular surface to dexamethasone. (**D**) Temporal course of wound healing after exposure of the ocular surface to mitomycin C.

nation of the applied dose and also transfer the test substance to the partner (control) globe.

Clearly, no tear-film buffering or lid-blinking action is possible in the *ex vivo* model, and it does not, therefore, precisely mimic the *in vivo* situation. Nevertheless, it furnishes an optimal and highly reproducible test algorithm for eye-drop application and for the control of experimental criteria. A few other *in vitro* models have been established to assess corneoepithelial wound healing. Tanelian and Bisla[15] used a setup involving rabbit corneoscleral explants mounted within a perfusion chamber, and Zieske et al. employed an organ-culturing technique.[16,17] But in both instances, the corneoscleral buttons were immersed in the culture medium, and swelling of the corneal stroma thus inevitably ensued. However, the influence of this phenomenon on wound healing was not independently assessed in these previous studies. Our investigation confirms that the cellular components essential for corneoepithelial wound healing are present in the *ex vivo* eye. Explanted porcine eyes can be maintained in a stable condition for up to 48 h without evident tissue changes, and this renders the model convenient for conducting experiments within the framework of a normal laboratory working schedule. The infusion of HEPES-buffered tissue-culture medium into the intact bulbus serves to maintain a physiological pH. Its delivery at a standard infusion height of 20 cm above the globe sustains the intraocular pressure, thus helping to preserve corneal transparency. The supply of antibiotic/antimycotic agents helps to control a possible growth of microorganisms, thereby contributing to the reproducibility of the results. Hence, this model is eminently suitable for assessing the influence of drugs on corneoepithelial wound healing during the course of a single day.

One possible concern may relate to the use of physiological saline rather than tissue-culture medium for the baseline measurements. But we deemed that the presence of nutrients would

have a positive influence on wound healing in our model, which was indeed the case (Fig. 1); and for this reason, tissue-culture medium served as a positive control, but not as a baseline standard. This permitted us to differentially assess the effects of the usually nutrition-free drug solvent and of nutritional factors on wound healing.

At this preliminary stage, we wished only to ascertain whether our *ex vivo* porcine model would be a useful one to assess the effects of novel drugs on corneoepithelial wound healing. Having established its sufficiency in this respect, we can now conduct a more sophisticated analysis, involving an assessment of cell metabolism, proliferation and apoptosis, and of cell-cell/cell-matrix contacts. It should be borne in mind that owing to he relatively small changes observed in lesion size, the outlined assay cannot yield information respecting the chronic toxicity of applied agents. For example, when applied at a concentration of 0.1 M, 3 drops of NH_4^+ exerted no untoward effects on wound healing. But clearly, this finding does not permit us to conclude that 0.1 M NH_4^+ can be used with impunity.

Using our model, we compared the effects of three different agents (three therapeutics and three controls) on corneoepithelial wound healing under identical conditions.

Cyclosporin A is a potent immunosuppressive drug, which is used systemically in ophthalmology to treat uveitis and to prevent corneal allograft rejection.[18] When applied topically, the known systemic side-effects of the drug are avoided. Nevertheless, local toxic effects on the corneal surface have been observed in clinical practice, especially after keratoplasty, when superficial punctate keratopathy frequently develops. However, in three reported animal studies, the topical application of cyclosporin A at 20 mg/mL (2%) for a period of several days had no impact on corneoepithelial wound healing.[19–21] The increased incidence of punctate keratopathy observed in humans who have undergone keratoplasty and been treated with cyclosporin A cannot, therefore, be clarified by the findings of these animal studies, but it can be explained by our own experiments. At concentrations of 0.1 mg/mL and 1.0 mg/mL, cyclosporin A had no effect on corneoepithelial wound healing in our *ex vivo* porcine model; but at 10 mg/mL, it tended to delay the onset of this response until after 22 h, without evident toxic effects (i.e., without eliciting an increase in lesion size). After penetrating keratoplasty, the cornea remains in a denervated state for approximately 1 year. This condition is reflected in a decrease in the mitotic rate of the corneal epithelium[22,23] and in an increase in the tissues' permeability to exogenous substances.[24] Because the cornea was not denervated in the above-cited animal studies, the absence of a cyclosporin A-induced effect on corneoepithelial wound healing is not surprising. In this respect, our *ex vivo* model resembles the clinical situation more closely.

For nonspecific suppression of inflammation, corticosteroids (e.g., dexamethasone) are the most effective drugs. These agents are widely used in ophthalmology, and because they are also applied to corneoepithelial lesions under certain circumstances, their effects on the ocular surface must be ascertained. Numerous studies have been conducted with this aim in view, but the findings are contradictory.[25,26] We applied three different concentrations of dexamethasone (0.1 mg/mL; 1.0 mg/mL; 10 mg/mL) to the ocular surface of our *ex vivo* porcine eyes, but in no instance did this agent influence the corneoepithelial wound-healing response within the time frame of the experiment (up to 26 h). This result accords with the findings reported by Srinivasan and Kulkarni.[27] These authors demonstrated that when applied three times daily at a dose of 1.0 mg/mL, dexamethasone decreased the rate of wound healing in rabbits only after complete, but not after partial, denudation of the corneal epithelium. Petroutsos et al. have reported the repeated application (6- or 16-times daily) of dexamethasone at a dose of 1.0 mg/mL to retard the healing of complete superficial corneoepithelial ulcers,[28] which accords with the results reported by Ho et al.[29] In another animal study, the treatment of alkali-induced corneoepithelial lesions with dexamethasone at a dose of 1.0 mg/mL four times daily for 1 week induced only a transient retardation of the wound-healing response, whereas treatment for 8 weeks (likewise at a dose of 1.0 mg/mL four times daily) elicited a more enduring effect.[30] In a further study, inhibition of corneal stromal wound healing, which was determined by measuring the intraocular pressure required to rupture the lesion, was first observed 6 d after treatment with dexamethasone, not before, and continued for at least 15 d.[31] The results of these studies indicate that the effects of dexamethasone depend upon the size and depth of the corneal defect, the frequency of drug

application, and the duration of the follow-up time, but not, apparently, on the dose administered. Furthermore, differences have been noted between animal species. Dexamethasone has been observed to delay the healing of corneoepithelial lesions in guinea pigs but not in rabbits.[19]

Mitomycin C is an alkylating agent with antineoplastic and antibiotic properties.[32] It is frequently applied to the ocular surface to control wound healing in the context of glaucoma-filtering surgery, to prevent the recurrence of pterygium, to forestall hazing during refractive surgery, and to treat intracorneoepithelial neoplasia.[33–35] In one *in vivo* study with rabbits conducted by Lai et al.,[36] the application of mitomycin C to corneoepithelial lesions at a dose of 0.2 mg/mL (initially for 12 h and then twice-daily for 5 d) did not retard the wound-healing response but did reduce subcorneoepithelial (stromal) haze during the 5-d follow-up period (with monitoring every 12 h). We analyzed the wound-healing response more minutely using different concentrations of mitomycin C. At the lowest dose (0.01 mg/mL), mitomycin C had no influence on corneoepithelial wound healing at 20 h and 22 h, but facilitated this response at 24 h and 26 h. At 0.1 mg/mL, this agent inhibited corneoepithelial wound healing at 20 h and 22 h, but not thereafter. At the highest dose tested (1.0 mg/mL), mitomycin C had a toxic effect, which was reflected in an increased lesion size from the onset (20 h) to the end (26 h) of the monitoring period. Ando et al have reported a similar dose-dependent retardation of corneoepithelial wound healing by mitomycin C in rabbits.[37] Hence, the choice of concentration is critical and should take into account the desired effect (i.e., the prevention of haze during refractive surgery or the inhibition of corneoepithelial wound healing in glaucoma-filtering surgery).

CONCLUSIONS

In summary, our *ex vivo* photorefractive, keratectomy-like porcine model for wound healing of the ocular surface furnishes us with a tool for assessing the influence of novel drugs on the stability and the repair of the corneal epithelium. Using a selection of test agents that are in current clinical use, we obtained reproducible quantitative information at different times and with different drug doses. The model is widely available, cheap, simple to establish, and yields useful data within the course of a normal laboratory working day. These favorable aspects render the model suitable for a broad application in the testing of novel drugs. Furthermore, its use may help to cut down on the resources and time invested in the experiments with living animals that must necessarily precede clinical trials. The inclusion of assays to assess qualitative aspects of corneoepithelial wound healing would be possible, but would render the model less simple, less cost-effective, and less widely applicable.

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Reprint Requests: *Justus G. Garweg, M.D.*
Department of Ophthalmology
Inselspital
University of Bern
3010 Bern
Switzerland

E-mail: justus.garweg@insel.ch

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