Concentration-Dependent Effects of Lidocaine on Corneal Epithelial Wound Healing

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Local anesthetic toxicity is a recognized clinical problem that has limited the use of topical corneal anesthetics for pain relief after corneal abrasion. Studies have shown clinically administered concentrations (0.5–2%) of local anesthetics impair corneal reepithelialization. Unfortunately, instillation of local anesthetic drops into an eye does not provide a measurable, steady-state concentration of drug. Thus, it has not been possible to evaluate whether there is an analgesic concentration of local anesthetic that does not impair corneal wound healing. Using the new in vitro rabbit cornea wound healing model, the effect of steady-state lidocaine concentrations on epithelial wound healing was examined. At lidocaine concentrations below 100 μg/ml, wound healing was not impaired. Higher concentrations (250–1000 μg/ml) resulted in dose-dependent impairment of epithelial wound healing. Combined with electrophysiologic evidence that corneal nerve injury discharge can be abolished by lidocaine concentrations less than 100 μg/ml, this research suggests that topical lidocaine in low concentration may be a safe topical corneal analgesic. Invest Ophthalmol Vis Sci 33:3029–3033, 1992

Local anesthetics can provide excellent corneal analgesia. Unfortunately, prolonged application of local anesthetics is associated with delay of corneal reepithelialization after wounding, altered lacrimation and tear film stability, corneal swelling, and disruption of epithelial cell motility. More recent studies have shown that even a single application of topical anesthetic can cause deleterious changes in the corneal epithelium. These clinical and laboratory studies use local anesthetic concentrations of 0.5–2% (5,000–20,000 μg/ml), which are in the range of currently used ocular formulations.

Recent electrophysiologic studies of corneal nerves have demonstrated that, after a corneal abrasion, tonic action potential injury discharge occurs that can be blocked with low steady-state concentrations of the local anesthetic lidocaine (ED50; 5.3 μg/ml). Similarly, clinical studies have shown that low plasma concentrations (2–5 μg/ml) of lidocaine relieve pain in humans. An additional benefit of lidocaine may be minimal corneal toxicity compared to other local anesthetics. These results suggest a potential to produce safe prolonged topical corneal analgesia with low dose (<100 μg/ml) lidocaine, a local anesthetic currently not used by ophthalmologists. To evaluate this hypothesis, the in vitro whole mount corneal wound healing model was used to study the effects of varying concentrations of lidocaine on corneal wound healing rates. Lidocaine was shown to produce dose-dependent effects on the rate of corneal epithelial wound healing.

Materials and Methods

Animal care and treatment in this study complied with the Institutional Animal Care Review Committee at Stanford University School of Medicine, and followed the ARVO resolution on the use of laboratory animals. New Zealand white rabbits, weighing 2–3 kg, were killed with an intravenous injection of Beuthanasia (pentobarbital/phenytoin; Schering Corp., Kenilworth, NJ) after anesthesia with an intramuscular injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). The eye was protruded and rinsed with corneal Ringer’s solution equilibrated with 95% O2/5% CO2 by bubbling with this gas mixture at 21°C. The cornea and a 2 mm rim of sclera were excised, and the iris and lens were removed. The cornea was mounted onto an in vitro whole mount perfusion chamber. Mounted corneas from the same animal then were placed into a common environmental chamber to ensure identical temperature and humidity conditions. The epithelial side of the cornea was maintained in a warmed, humidified air environment. The endothelial side of one cornea was perfused with Gibco’s (Grand Island, NY) Medium 199 and 50 μg/ml gentamicin equilibrated with 95% O2/}

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5% CO₂ and brought to a pH of 7.4. The other cornea received the same media containing lidocaine at one of the following concentrations: 1000 μg/ml, 500 μg/ml, 250 μg/ml, or 100 μg/ml. Corneas were maintained at 35°C and their intraocular pressure was maintained at 15 mmHg.

**Corneal Wounding**

A Bard-Parker No. 15 blade was used to create an anterior keratectomy subepithelial wound. A 5 mm circular trephine mark was made in the center of the cornea to mark the wound area, and the epithelium, basement membrane, and part of the anterior stroma were scraped away. Wounding was confirmed by staining with 0.5% fluorescein.

Rates of wound closure were monitored by staining with fluorescein every 5 hr and recording the average radius of the remaining wound. Corneas were allowed to heal until fluorescein failed to stain the cornea, which was reported as the time of wound closure. The quality of reepithelialization was evaluated by stereomicroscopic (M3Z; Wild Leitz, Heerbrugg, Switzerland) visualization after fluorescein staining. Data were statistically analyzed using repeated measures analysis of variance and the methods of Crosson, Klyce, and Beuerman.²²

**Histology**

Corneal wound production was confirmed by prior wound technique histologic analysis. After wound closure, the corneas were rinsed in corneal Ringer’s solution and fixed in 10% buffered formalin fixative for histologic studies. The corneas then were stained with hematoxylin-eosin/phloxine counterstain as well as periodic acid-Schiff stain to highlight the basal lamina. Corneas were sectioned (6 μm) and mounted with Permound (Fisher Chemical, Fair Lawn, NJ). Sections were analyzed with a Nikon Diaphot inverted microscope.

**Results**

Corneal epithelial wound healing was not altered at a lidocaine concentration of 100 μg/ml (P > 0.5), the lowest concentration tested (Figs. 1A and 2). Control and 100 μg/ml corneas healed in a circularly symmetrical fashion and remained transparent throughout the healing process (Fig. 1A). Control corneas healed at a rate of 37 ± 4 μm/hr (mean ± standard error of the mean, n = 5), while corneas exposed to 100 μg/ml of lidocaine healed at a rate of 35 ± 1 μm/hr (mean ± SEM, n = 5). The initial latency period was minimal for control and lidocaine-treated corneas, lasting approximately 5 hr. After the initial latency period,
Fig. 2. The average decrease in wound radius at specific times after wounding for control corneas (n = 5; circles) and paired corneas treated with 100 μg/ml lidocaine (n = 5; triangles). Both corneas remained transparent throughout the healing process and healed at similar rates—35 ± 1 μm/hr (mean ± standard error of the mean) for controls and 37 ± 4 μm/hr for the 100 μg/ml lidocaine-treated corneas. Complete wound closure occurred in approximately 67 hr for control and 100 μg/ml-treated corneas.

healing proceeded in a linear fashion. This slope was used to calculate the wound healing rate. Complete wound closure occurred in 67 ± 7.6 hr for control and 100 μg/ml lidocaine-treated corneas (Fig. 2). Corneas treated with 100 μg/ml lidocaine were histologically similar to control corneas (Figs. 3A and B). In both cases, a single layer of epithelial cells had migrated over the wounded area, and the epithelium from undamaged areas of the cornea had thinned. Thickness comparisons revealed no increase in corneal thickness between control and lidocaine-treated corneas; the endothelium appeared normal, and the epithelium appeared normal for both.

When the concentration of lidocaine was increased to 250 μg/ml, inhibitory effects on epithelial wound healing were observed (Fig. 1B). The linear rate of wound closure for the 250 μg/ml lidocaine-treated corneas was 28 ± 5 μm/hr (mean ± SEM, n = 5) compared to control healing at 37 ± 4 μm/hr (Fig. 4). After initially healing in a symmetric fashion for 60 ± 5 hr, wound healing was arrested and followed by an increase in wound size (Fig. 1B). The epithelial layer of corneas maintained for 75 hr was fragile. The 250 μg/ml lidocaine-treated corneas lost transparency approximately 32 ± 15 hr after wounding. Some corneas (n = 3) showed a greater latency period (lasting approximately 13 ± 4.4 hr) than control and 100 μg/ml lidocaine-treated corneas. Histologic analysis revealed that the epithelial layer covering the damaged areas contained gaps and that central areas of the cornea remained denuded (Fig. 3C).

Corneas treated with 500 μg/ml (n = 5) and 1000 μg/ml lidocaine (n = 5) did not heal. At these higher concentrations, lidocaine-treated corneas lost transparency more quickly (11 ± 5.9 hr for the 500 μg/ml corneas and 6 ± 2.2 hr for the 1000 μg/ml corneas). These corneas became progressively more edematous and cloudy until the entire cornea was opaque (Figs. 1C and D). Corneas treated with 500 μg/ml lidocaine had increased latency periods of 13 ± 2 hr. The wound healing rate decreased for these corneas to 22 ± 9 μm/hr until wound size began to increase 57 ± 5.7
average of 3.9 mm in radius. In the 1000 μg/ml lidocaine-treated cornea measured an increase slightly (28 ± 5) for corneas treated with 250 μg/ml lidocaine (n = 5; upside down triangles). These corneas began to reverse the wound healing process approximately 60 hr after wounding, whereas corneas treated with 500 μg/ml lidocaine (n = 5; diamonds) began this trend sooner. Wound healing rates slowed to 22 ± 9 μm/hr for these corneas. Corneas treated with 1000 μg/ml lidocaine (n = 5; triangles) showed the greatest delay in epithelial migration and the earliest extension of wound margins (44 ± 4.4 hr).

hr after initial wounding (Fig. 4). After 60 hr, the entire corneal epithelial defect stained with fluorescein. At the time when wound closure was complete in the control or 100 μg/ml corneas (approximately 70 hr), the 500 μg/ml lidocaine-treated cornea measured an average of 3.9 mm in radius. In the 1000 μg/ml lidocaine-treated corneas, wound healing was even more impaired. Wound size remained approximately 5 mm in diameter for 28 ± 9.7 hr and then increased to include the entire corneal surface within 44 ± 4 hr (Fig. 4). Histologic analysis of the 500 and 1000 μg/ml corneas showed that corneal thickness was increased at both concentrations, with intrastromal edema occurring at 1000 μg/ml of lidocaine (Figs. 3D and E). Reepithelialization was completely halted at these lidocaine concentrations.

Discussion

The results of this study establish that low analgesic concentrations of lidocaine (≤100 μg/ml) do not impair corneal epithelial wound healing or corneal transparency. Previous in vivo studies1-5,14,16 were not able to examine the effects of these low concentrations of lidocaine because steady-state delivery of local anesthetic solutions in the in vivo eye is relatively impossible. These in vivo ocular studies looked at clinical concentrations of anesthetic (5,000–20,000 μg/ml, applied with an eye dropper a specified number of times daily) and concluded that continuous application of local anesthetic should not be used on normal or injured corneas. These clinical observations were confirmed by this investigation. When concentrations of 250 μg/ml or greater were applied, normal wound healing was significantly impaired. In addition to impaired wound healing, the stromal layer of the cornea swelled and transparency was lost, especially at 5000 μg/ml (0.5%) and 10,000 μg/ml (1.0%) concentrations. Clinical local anesthetic abuse (0.5% proparacaine hydrochloride) also is associated with epithelial defects and stromal haze23 comparable to that seen in the in vitro rabbit cornea preparation.

The mechanism or mechanisms by which local anesthetics negatively alter wound healing is not known. Local anesthetics have been shown to disrupt the surface microvilli of epithelial cells, decrease mucous adherence, and shorten tear breakup time.7-10 These effects, coupled with inhibition of the normal corneal tearing and blink reflex, may lead to secondary corneal injury because of corneal drying. In the in vitro rabbit cornea whole mount preparation, the control and experimental corneas were maintained at identical temperature and humidity conditions that kept the epithelium moist at all times. These experiments suggest that the local anesthetic effects observed upon corneal wound healing likely result from direct toxicity to the cornea.

Direct epithelial toxicity in response to topical anesthetics has been reported in vivo1,4,5,12,16 as well as in vitro.15 The experiments of Dass et al15 demonstrate that proparacaine at concentrations above 300 μg/ml result in alteration of the corneal epithelial actin cytoskeleton. At 300 μg/ml, epithelial cell spreading and migration was impaired; at 1650 μg/ml, all of the epithelial cells were rounded and detached. Higbee and Hazlett13 have shown 5000 μg/ml of benoxinate, proparacaine, and tetracaine decrease corneal epithelial injury, myosin, and calmodulin. This investigation did not look at the effect of lidocaine on epithelial cytoskeletal proteins. However, it is interesting that at comparable concentrations, lidocaine and proparacaine produce equivalent impairment of epithelial cell migration, suggesting a possible effect on cytoskeletal proteins. At a molecular level, local anesthetics have been shown to inhibit protein kinase C from rat brain.23 Inhibition of corneal epithelial wound healing by protein kinase C inhibitors has been demonstrated24 in rat corneas. These findings suggest that inhibiting the phosphorylation of a critical protein, possibly involved in cytoskeletal contraction, via protein kinase C inhibition, may be the molecular
mechanism of local anesthetic impairment of wound healing.

Low (<100 μg/ml) concentrations of local anesthetics do not completely block peripheral nerve conduction, nor do they provide anesthesia when applied topically. However, recent experiments looking at corneal nerve electrophysiology have shown that tonic corneal nerve injury discharge can be suppressed by low concentrations of lidocaine without impairing electrically evoked action potential discharge. In humans, acute and chronic pain can be suppressed by low (<5 μg/ml) plasma concentrations of lidocaine without systemic toxicity. The in vitro experiments suggest that local anesthetics at analgesic concentrations of ≤100 μg/ml may be safe for continuous corneal administration and provide corneal analgesia after corneal abrasions or corneal refractive procedures. Further in vivo and clinical studies need to be conducted to establish the efficacy of such therapy before it is implemented.

Key words: cornea, epithelium, in vitro, lidocaine, wound healing

References