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Effects of morphine on corneal sensitivity and epithelial wound healing: implications for topical ophthalmic analgesia

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Abstract

Studies were conducted to examine the analgesic and toxic effects of topical morphine on corneal abrasion. For the toxicity study, rabbits were anaesthetised and epithelial cells were removed from the cornea and limbus. Animals were randomised and treated topically as follows: (1) saline (control); (2) morphine sulphate (MS, 0·5%); and (3) proxymetacaine hydrochloride (proparacaine) (PH, 0·5%). Two drops of the solution were instilled in the eyes at 4 hour intervals for 6 consecutive days and the progression of corneal wound healing was assessed. Results showed that repeated topical MS had no adverse effects on corneal wound closure. The rates of wound healing were similar in both saline and MS treated groups. Eyes treated with MS showed wound closure in a symmetrical fashion starting on day 2 following abrasion. The progression of epithelial wound healing was completed by day 4 in one eye, by day 5 in three eyes, and by day 7 in five eyes. In contrast, repeated topical PH application delayed corneal wound closure. Eyes treated with PH showed signs of corneal wound closure on the third day, but only two eyes out of six had completed wound closure by the eighth day after corneal abrasion. In a subsequent masked study, the analgesic efficacy of topical MS was assessed in seven patients with unilateral corneal abrasion. In all cases, a baseline response was first established. Subsequently, saline was instilled in both eyes and the patient's corneal response to pain pressure was determined 10 and 20 minutes later. Finally, MS was applied and the analgesic effect on the cornea was assessed. Results showed that saline had no effect compared with the baseline response. In contrast, MS showed an analgesic effect as early as 10 minutes after application in the eye with corneal abrasion. MS showed an analgesic efficacy of 4·3-fold and 5·5-fold greater than the baseline or saline on the eye with corneal abrasion. However, MS had no analgesic effect on the intact cornea. Collectively, these data indicate that opioids do have a desirable analgesic property without irritating or causing any adverse effect on ocular structures.

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Currently, ophthalmic analgesia is obtained by using topical anaesthetic agents, including tetracaine, procaine, benoxinate, proxymetacaine (proparacaine), etc.[1] These ocular formulations at therapeutic doses of 5000 to 20 000 μg/ml (0·5% to 2%) exhibit anaesthetic properties by inhibiting the conduction of corneal nerve impulses.[2] Unfortunately, repeated or prolonged application of ophthalmic anaesthetics has been associated with deleterious effects on the corneal epithelium.[3-6] Furthermore, recent studies have demonstrated that even a single dose of topical anaesthetic can cause severe toxicity to the corneal epithelium.[7] Major toxic effects of these drugs include: (1) inhibition of corneal re-epithelialisation[3,4] that may result in sloughing of the corneal epithelium; (2) alteration in lacrimation and mucus adherence[8,9] causing a decrease in stability of the precorneal tear film; (3) increase in corneal permeability and swelling[10] which results in loss of corneal transparency; and (4) altering corneal epithelial cytoskeletal elements (actin, myosin), which causes disruption of cell motility.[5,6] Other adverse effects include allergic dermatitis in sensitive patients. Thus, the toxic effects exhibited by these drugs severely limit their application as topical ophthalmic analgesics. Following application of topical anaesthetics, the loss of corneal sensitivity is so profound that some patients inadvertently injure their corneas without being aware of the extent of self injury. An alternative but effective topical analgesic with minimal or no toxic effect is therefore desirable. Certain ocular conditions, diseases, and injuries would be treated if a safer analgesic was available. An ideal formulation should be composed of an effective analgesic which does not cause any adverse effects or permanent damage to ocular structures. Such a formulation may have potential application for: (1) injuries to the eye causing damage to the corneal epithelium and conjunctiva, such as traumatic corneal abrasion, penetration, perforation, acid and alkali burn; and (2) diseases causing dry eye syndrome and subsequent localised or diffused corneal epithelial cell damage, such as keratoconjunctivitis sicca.

For these reasons, development of a safer and effective topical formulation for ophthalmic analgesia is warranted. These studies were designed to evaluate the analgesic effects of an opiate agonist, morphine, on corneal sensitivity in patients, and to compare its potential toxicity with proxymetacaine hydrochloride (PH) on corneal epithelial cells in the rabbit. Opioid agonists have been shown to exhibit potent antinociception that is mediated both by the central nervous system and peripheral tissues.[11] We report that topical application of morphine sulphate (MS) solution at low concentrations resulted in effective analgesia in patients with corneal abrasion as early as 10 minutes after local application. The analgesic formulation did not adversely affect

corneal wound closure, as examined in a corneal abrasion and healing model. To our knowledge, the effects of an ophthalmic opiate as a topical or local analgesic for corneal and conjunctival pain therapy have never been described.

Materials and methods

PREPARATION OF MORPHINE SOLUTION

Morphine sulphate (MS) was dissolved in normal saline as a concentrated solution. Then, appropriate dilutions were made from the stock solution in order to prepare a morphine concentration of 5 mg/ml (0·5%) and 0·5 mg/ml (0·05%). The 0·5 mg/ml concentration of morphine was chosen for the analgesic study because previous studies have shown it to be sufficient to cause suppression of pain pressure in animal and pain response in humans.[11] The pH of the solution was adjusted to approximately 7·2 with the addition of hydrochloric acid and/or sodium hydroxide solution. The osmolarity was also adjusted to 300 mosmol with the addition of sucrose or glucose. Immediately before each study, the morphine solution was sterilised by filtering it through a 0·2 μm filter in a sterilised test tube. The solution was drawn into a syringe and used as needed. The stability study (data not shown) indicated that there was no detectable morphine degradation or change in colour when the solution was protected from light and stored at −20°C or 4°C for over a month.

TOXICITY TEST IN ANIMALS

The eyes of healthy pigmented rabbits weighing 1·5–2·2 kg were examined by slit-lamp biomicroscopy. Animals were anaesthetised with intramuscular ketamine hydrochloride (Ketaset, Aveco, Fort Dodge, IA, USA) 50 mg/kg and xylazine (Rompum, Haver, Shawnee, KS, USA) 5 mg/kg body weight. Topical corneal anaesthesia was avoided in all animals in order to prevent epithelial cell toxicity from the anaesthetic agent. Under direct visualisation through a microscope, corneal epithelial cells were removed in one eye of each animal using a No 15 Beaver blade.[12] The cell scraping was done moving from the inside outward and including the limbus, avoiding damage to the conjunctiva while the basal lamina was left intact. In order to ensure thorough de-epithelialisation, one drop of fluorescein 1% was instilled on the corneal surface, and further scrapes were made when necessary to remove all the corneal epithelium. After the de-epithelialisation process, all eyes were gently washed with saline to remove excess fluorescein and to verify the complete corneal abrasion.

For drug treatment, animals were randomly divided into three treatment groups, as follows: (1) saline solution (control, n=eight rabbits), (2) morphine formulation 0·5% (n=six rabbits), and (3) proxymetacaine hydrochloride 0·5% (n=six rabbits) (Alcaine, Alcon Laboratories, Fort Worth, TX, USA, positive control). Only one eye of each animal was included in the study. Eyes in each group were instilled in a blind fashion with two drops of the corresponding solution at 4 hour intervals for 6 consecutive days. To avoid possible interference with the healing process, topical prophylactic antibiotics were not used. Wound healing (corneal wound closure) was then assessed on days 2, 3, 4, 5, 6, 7, and 8 following corneal abrasion using fluorescein staining with Fluor-I-Strips (Wyeth-Ayerst Laboratories, Philadelphia, PA, USA). Corneal wound closure was monitored by clinical examination as well as wound surface area measurements. Measurements of stained corneas were made by slit-lamp, using a ruler to determine the diameters and/or sides of the remaining lesions (two to five measurements, depending on the geometric shape of the lesion; for example, a circle, a square, a triangle, etc) and thereafter the stained areas were calculated using an appropriate mathematic formula. Four eyes in the control group and one eye in each of the experimental groups were excluded from the experiment due to infection.

ANALGESIC TEST IN PATIENTS

This study was designed to examine the analgesic efficacy of the morphine formulation in human subjects with post-surgical corneal abrasion when the drug was applied topically to the eye. Informed consent was obtained from seven patients who had unilateral corneal abrasions during intraocular surgery (vitrectomy). In all cases, three measurements were made in the following fashion: firstly, a baseline measurement (corneal sensitivity response) was performed by determining the responses of denuded eye with abrasion and the contralateral eye without abrasion to a standard pain pressure, using a Cochet-Bonnet aesthesiometer (Luneau, Paris, France) instrument, without applying any drug. Measurements were made beginning with the nylon monofilament (0·12 mm diameter) fully extended. The tip was applied perpendicularly to the corneal surface and gently pressed until the fibre's first visible bending. The length of the fibre was gradually decreased until a blink reflex and/or a verbal pain response were observed. The length was then recorded in millimetres. Secondly, a saline solution (two drops) was instilled in both eyes; 10 and 20 minutes later the response of the patient's corneas to pain pressure was determined as described above. The repeated measurements were made to rule out the placebo effect. Finally, two drops of the morphine formulation (0·05%) were instilled in both eyes and the analgesic effect on the cornea was assessed. The clinician responsible for evaluation of the pain response was not informed of the nature of the drugs being tested.

Data analysis

The analgesic efficacy of MS was assessed in patients with corneal abrasion and without abrasion. The significance of the differences between the mean values of drug treated eyes, the control before the treatment, and the contralateral eye was determined by one way analysis of variance (ANOVA) and Scheffe F test. The level of probability for all tests was $p<0·05$.[13]

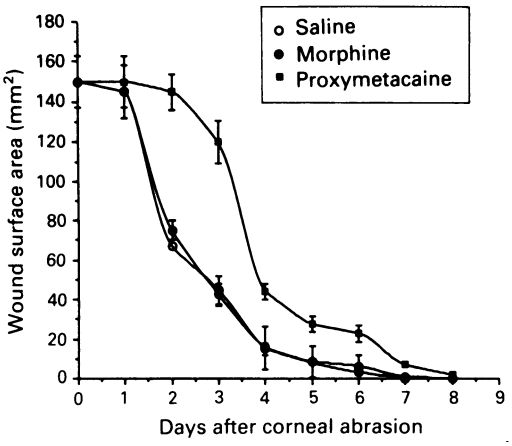

Figure 1 Toxicity study of corneal wound healing. (-⊖-) indicates the effect of saline on corneal wound healing; (-●-) indicates the effect when morphine (0·5%) was applied to the cornea; and (-■-) indicates the effects of proxymetacaine (0·5%). (Represented is the mean with SEM for n=6–8 at each time point. When not visible, the error bars are smaller than the symbols.)

Results

The results of the toxicity study of corneal wound healing are shown in Figure 1 and Table 1. As shown in Figure 1, repeated topical application of the MS formulation had no adverse effect on corneal wound closure. The rates of wound healing were similar in both saline and MS treated groups. However, 50% of the eyes (four out of eight eyes) developed infection and thus were eliminated from the study. Eyes treated with MS formulations started to show wound closure on day 2 and some eyes had completed wound closure 4 days following corneal abrasion. By day 4, one eye was healed; by day 5, three eyes demonstrated wound closure; and by day 7, five eyes were healed. Only one eye treated with MS did not show complete wound closure, by the last observation time point, as a result of infection. In contrast, repeated application of PH significantly delayed corneal wound closure. Eyes treated with PH began to show corneal wound closure on the third day and in some eyes wound healing was complete 8 days following corneal abrasion. The time course study showed that only two out of six eyes treated with PH had completed closure of the corneal epithelium by 8 days (the last time point investigated). Interestingly, only one of the remaining four eyes in the PH group, which did not show complete epithelial regeneration by the last observation time point, developed infection.

Results of the analgesic study in patients with corneal abrasion are shown in Table 2. The analgesic efficacy of MS on the corneal sensitivity is expressed as pressure in g/mm². Results show that instillation of saline solution into the eyes had no analgesic effects, as expected. The analgesic effect, as determined by corneal sensitivity to saline at 10 and 20 minutes, was similar to the baseline response for both eyes. In contrast, topical application of two drops of MS formulation (0·05% solution) to the denuded corneas exhibited a dramatic analgesic effect as early as 10 minutes after administration. The magnitude of pain suppression by the MS formulation was such that much greater pressures were required to obtain a corneal sensitivity response. The MS formulation showed an analgesic efficacy on the denuded corneas of 4·3-fold and 5·5-fold greater than the baseline or saline at 10 and 20 minutes, respectively. Interestingly, the magnitude of the analgesic response observed 20 minutes after MS treatment was greater than the response observed after 10 minutes, indicating a time dependent analgesic effect by MS. Thus, it seems likely that the maximum analgesic effect of MS occurs at least 20 minutes following topical application. Topical application of the MS formulation into the normal contralateral eye did not produce an analgesic effect at all.

Table 1 Progression of corneal epithelial regeneration following mechanical epithelial cell removal

Drug	Dose (drops)	Days after drug treatment 1	2	3	4	5	6	7	8
Saline	2	0/8*	0/8	0/8	1/8†	3/8‡	3/8**	4/8**	4/8**
Morphine	2 (0·5%)	0/6	0/6	0/6	1/6	3/6	4/6†	5/6†	5/6†
Proxymetacaine	2 (0·5%)	0/6	0/6	0/6	0/6	0/6	0/6	0/6	2/6†

*Number of eyes exhibiting complete healing of the cornea/total number of eyes per group.
†One eye developed corneal infection.
‡Three eyes (three animals) developed corneal infection.
**Four eyes (four animals) developed corneal infection.

*Table 2 Analgesic effect of morphine sulphate (0·05%) on the sensitivity of patients' corneas with abrasion expressed as pressure (g/mm²)**

Drug	Dose (drops)	Time after topical drug treatment (minutes) 1	10	20
None	Baseline	1·95 (0·20)	–	–
Saline	2 (0·9%)		1·73 (0·42)	1·68 (0·72)
Morphine	2 (0·5%)		7·69 (2·16)†	9·84 (1·00)†

*Values are the mean pressures in g/mm² (SEM) for seven patients.
†Indicates difference from the saline and baseline values.

Discussion

The results of this study demonstrated that when low dose MS was applied topically to eyes with corneal abrasions, a pronounced and relatively rapid analgesic effect occurred. The fast analgesic response indicates that MS exerts its effects locally on the ocular structures (cornea and conjunctiva) primarily through opioid receptor mediated mechanisms. This interpretation is supported by the fact that several recent studies have indicated that some inflamed tissues contain opiate receptors, which may be directly involved in producing the analgesic response to opiate application by interacting with the drug locally in the tissue.[14,15] Interestingly, topical MS did not produce any analgesic effect on the intact cornea.

The only indication that opiates may have receptors and perhaps a physiological effect on the surface structures of the eye comes from the findings of Fanciullacci *et al.*[16] Their findings have shown that when an opiate antagonist, naloxone, was applied topically to one eye of a morphine dependent subject (a conjunctival test model for morphine addiction), the opiate antagonist caused pupillary dilatation in the same eye.[16] Numerous other studies have examined the effects of opiates on pupillary diameter following intramuscular, intravenous, subcutaneous, or oral administration.[11] Miosis or pupillary constriction is a consistent effect of opiates and as there is an excellent correlation between the potency of analgesics in producing miosis and generalised analgesia, the miosis phenomenon has been utilised as a bioassay test for studying the time course and relative potency of opiate analgesic drugs in humans.[11] To our knowledge, the analgesic effects of morphine or other opiate

agonists as topical ophthalmic analgesics have not been previously reported.

In the past, morphine and other opiates used as analgesic agents have been administered systemically, including orally, subcutaneously, intramuscularly, and intravenously. The analgesic response experienced following the use of opiates is believed to be the result of opioid receptor activation in the central nervous system.[11] This theory, that opiates produce their effects by interacting with receptors, developed from observations that specific structural and stereochemical requirements are necessary for their analgesic action. The detailed pharmacological and molecular mechanisms of opioids have recently been uncovered.[14 15 17] The existence of multiple opioid receptor types and subtypes has been suggested, based on the relation between the molecular structure of opiate drugs and their analgesic effect.[14 15] Thus, the discovery of opiate receptors in the central nervous system intensified the search for endogenous opioids as well as attempts to demonstrate the presence of opiate receptors in the peripheral nerve terminals.[15] Several recent studies have determined that opiate agonists exhibit peripheral analgesic effects in inflammed tissue of animals,[17 18] and that the antinociceptive effects of opiate μ and κ agonists are enhanced by peripheral opioid receptor specific mechanisms.[15] Furthermore, in a double blind clinical trial, the analgesic efficacy of a low dose aqueous morphine solution was investigated.[19] When morphine was applied locally inside the joints following knee surgery, it significantly reduced pain scores, most probably because of local activation of opioid receptors that reached maximal effectiveness in 3 to 6 hours.[19] However, when morphine was applied to intact rabbit cornea (without epithelial cell abrasion or inflammation), the opiate did not have any inhibitory effect on the standard pain pressure produced by the Cochet-Bonnet aesthesiometer instrument (our unpublished observations). These findings are in agreement with previous studies indicating that opioid receptors mediate antinociception in inflammatory and/or stress situations.[14 15]

The lack of adverse effects of MS on the ocular structures, as assessed in the epithelial wound closure study, warrants further investigation and evaluation of these agents as ophthalmic analgesics. Our experimental observations showed no apparent sign of toxicity when compared with the control (saline solution). The data support the idea that a desired local analgesia can be achieved by these agents without impairing the normal function of the eye. The major dose dependent side effects of morphine administered systemically for postoperative pain are respiratory depression and urinary retention.[11] These problems would be minimal or non-existent if the drug were applied topically in the eye at such low doses.

An intriguing observation made in the corneal wound healing study was the fact that more rabbits' eyes in the control group (saline treated) developed infection during the course of corneal wound closure. We observed 50% (four out of eight eyes) infection in the control group.

The 0·5% Alcaine (proxymetacaine hydrochloride) solution used for these experiments contains 0·01% benzalkonium chloride as a preservative. Because these substances have been shown to be able to change the corneal epithelial permeability,[3] the delayed wound closure in this group of animals might have been caused by the presence of this preservative. The morphine solution did not contain a preservative.

Collectively, these results demonstrate that a topical formulation of MS provides effective analgesia without any adverse effects on the corneal epithelium and without having a profound anaesthetic property. Re-establishment of corneal sensitivity once the corneal defect is healed would prevent inadvertent self injury. This topical analgesic can be used for temporary relief of pain in patients having corneal and conjunctival diseases or injuries.

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