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Inhibition of Corneal Epithelial Cell Migration by Cadmium and Mercury

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Cadmium and mercury are significant aquatic pollutants which can have direct toxic effects on aquatic organisms and can affect humans through their presence in the water supply and the food chain. Cadmium is known to inhibit calmodulin and calcium-dependent processes (Cheung 1984) and is also a teratogen (Ferm 1971). Mercury, especially in its organic form, methyl mercury (MeHg), is a neurotoxin and a nephrotoxin. It inhibits sulfhydryl-dependent enzymes, mitosis, and cell migration (Clarkson 1987; Miura and Imura 1987; Sager 1988; Choi et al. 1981).

The above observations concerning effects of cadmium and mercury on calcium metabolism and cell migration suggested that these metals might also have inhibitory effects on migration of epithelial cells. Epithelial cell migration is important in development and in wound healing responses. In the present study the corneal epithelium of the eye is used as a model for the study of toxicity of cadmium and mercury to epithelial cells.

The corneal epithelium is a barrier to movement of water and pathogens into the stroma of the cornea. When the epithelium is abraded, the cells at the wound edge migrate across the exposed basement membrane to re-establish the epithelial barrier. The rate at which the abrasion is covered by cells is easily measured and this process of corneal epithelial healing is commonly used in ocular pharmacology and toxicology to determine effects of topical ophthalmic drugs and chemical substances on cell migration (Ubels et al. 1982).

In a previous comparative study of corneal healing in fish, we observed that corneal epithelial healing occurs very rapidly in vivo in the marine teleost Myoxocephalus octodecimspinosus (longhorn sculpin) with a 6-mm diameter wound closing in about 12 h compared to 2-3 days for a similar size wound on the mammalian cornea (Ubels and Edelhauser 1982). This rapid healing which permits prompt restoration of the epithelial barrier is apparently an adaptation -

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to the large ionic and osmotic gradients between the environment and the intraocular fluids of the fish (Ubels and Edelhauser 1987). These observations suggested that epithelial healing in the sculpin cornea might be a useful model in aquatic biomedical toxicology if an in vitro method for measurement of healing rates could be developed. In this report we demonstrate that sculpin eyes maintained in short-term organ culture have a rapid corneal epithelial healing response and that this model can be used to demonstrate the toxic effects of heavy metals on epithelial cell migration.

MATERIALS AND METHODS

Longhorn sculpin (Myoxocephalus octodecimspinosus) weighing 150±6 g were taken from the Gulf of Maine and maintained in tanks of 15°C flowing seawater at the Mount Desert Island Biological Laboratory, Salsbury Cove, Maine.

The fish were doubly pithed and a circular area of the corneal epithelium 6.5-7.5 mm in diameter was removed from both eyes by application for one minute of a heptanol-soaked Whatman number 1 filter paper disc (Cintron et al. 1979). This was followed by vigorous rinsing with seawater and removal of the damaged cells with a cotton swab. Both eyes were then removed intact from the fish.

One eye was mounted in a Lucite chamber filled with flowing seawater (2 mL/min) at 14-16°C. This eye served as a control and under these conditions normal corneal and lens transparency were maintained for at least 12 h. The contralateral eye was mounted in an identical chamber and superfused with seawater containing a compound to be studied for effects on cell migration. The chambers could hold the eyes of up to eight fish simultaneously and this arrangement allowed comparison of healing rates on a paired basis.

Corneal epithelial healing rates were measured as previously described (Ubels et al. 1982). Briefly, the wounds were stained with sodium fluorescein immediately after wounding and at 3-h intervals until wound closure. The wounds were photographed and wound areas and diameters were measured on the photographs using the Sigma Scan digitizing program (Jandel Scientific, Corte Madera, California). Wound healing rates (mm^2/h) were determined by linear regression. The migration of the epithelial wound edge was also monitored directly in some experiments by time-lapse video microscopy, and cell migration rates were determined from the video recordings. Healing rates were compared by the paired t-test.

The compounds investigated for effects on cell migration were $CdCl_2$, $HgCl_2$ (Sigma Chemical Company, St. Louis, Missouri), methyl mercuric chloride (K & K Laboratories, Cleveland, Ohio), the calmodulin inhibitor W7, and the microfilament inhibitors cytochalasin B and D (Sigma). The compounds were added to the seawater flowing through the experimental chamber at the concentrations indicated in the figures.

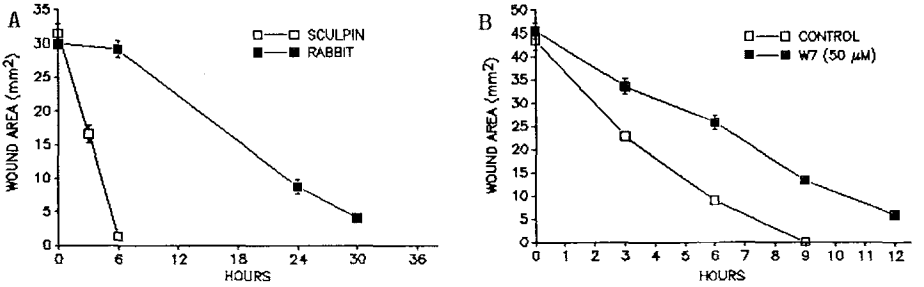

Figure 1. A. Comparison of corneal epithelial healing in the sculpin (in vitro) and the rabbit (in vivo). B. Inhibition of corneal epithelial healing in the sculpin by the calmodulin inhibitor, W7. Mean±SE, n = 5.

RESULTS AND DISCUSSION

As predicted based on in vivo data, corneal epithelial wounds on sculpin eyes healed rapidly in vitro with cells migrating to cover the abraded area at a mean rate of 5.2±0.15 mm^2/h for all control eyes in this study (n = 72). A healing curve for one group of five eyes is shown in Figure 1A, demonstrating that a wound with an initial diameter of 6.5 mm closes in slightly more than 6 h post wounding at a rate of 5.0 mm^2/h. As determined by video microscopy, the advancing edge of epithelial cells migrated at 0.4-0.5 mm/h. In contrast, previous work (Ubels et al. 1982; Matsuda et al. 1985) has shown that a similar wound on the rabbit cornea heals at about 0.9 mm^2/h (Figure 1A). The cells migrate at 0.05-0.06 mm/h (Matsuda et al. 1985; Crosson et al. 1986) and the wound closes at about 36 h. This rapid healing response and ease with which sculpin eyes could be maintained in vitro confirmed that this model would be well suited for studies of mechanisms of corneal wound healing and for toxicologic studies of epithelial cell migration. The metabolic or mechanistic differences between the fish and mammalian corneas which cause this striking difference in cell migration rates have not been determined.

To verify that the sculpin cornea would respond to known inhibitors of corneal epithelial healing (Soong and Cintron 1985) corneas were exposed to the calmodulin inhibitor, W7, and to the microfilament inhibitors, cytochalasin B (5 μg/mL) and cytochalasin D (1 μg/mL). W7 reduced the healing rate to 3.3±0.18 mm^2/h compared to a control rate of 5.8±0.24 mm^2/h (Figure 1B). The cytochalasins completely inhibited cell migration. When the corneas were returned to normal seawater after exposure to cytochalasin D for 1.5 h, cell migration resumed. These data confirm the calcium and calmodulin dependence of corneal epithelial cell movement in the sculpin as well as the role of microfilaments in this process.

Exposure of corneas with wounds 7 mm in diameter to $CdCl_2$ for 12 h resulted in a dose-dependent inhibition of cell migration. At 10^{-7} M no inhibition of healing was observed while at 10^{-5} M the healing rate was reduced to 2.43±0.40 mm^2/h compared to a control

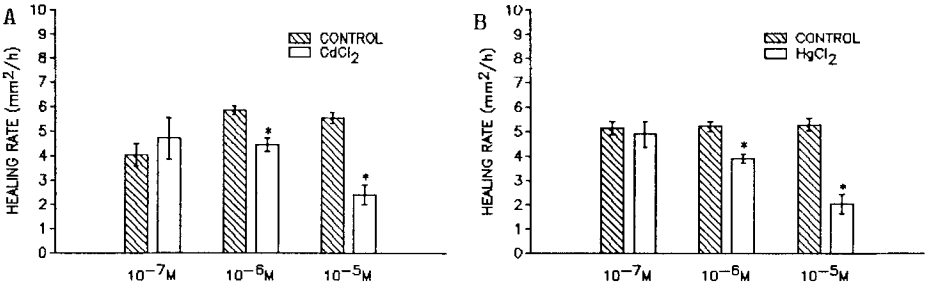

Figure 2. Effect of (A) cadmium and (B) inorganic mercury on corneal epithelial wound healing. * significantly different than paired control, $p \leq 0.05$. Mean±SE, n = 5.

rate of 5.57±0.23 mm^2/h (Figure 2A). Cell migration stopped after 9-h exposure to 10^{-5} M $CdCl_2$. It was determined by video microscopy that 10^{-5} M $CdCl_2$ reduced the cell migration rate to 0.35±0.01 mm/h from a control rate of 0.46±0.02 mm/h.

The time course of the effects of Cd^{2+} was also studied. In these experiments corneas were wounded and mounted for observation by video microscopy. The wounds were allowed to heal in normal seawater for 3 h at which time 10^{-5} M $CdCl_2$ was added to the superfusate. When the cadmium was added, the wounds were about 50% healed (as illustrated in Figure 1) and healing continued at the normal rate during exposure to Cd^{2+} until wound closure at 6 to 7 h (data not shown). This suggests that Cd^{2+} interferes with processes that initiate wound healing and that it is not able to stop a cell which has begun to migrate.

The effect of cadmium observed in this study may be related to its effects on calcium fluxes in the cell. Other investigators have shown that Cd^{2+} binds to calmodulin, inhibiting normal intracellular fluxes of calcium in vivo and in vitro (Cheun 1984; Vig et al. 1989). Cadmium also inhibits Ca^{2+}-ATPase in intestinal epithelium and brain. Calcium is essential to processes involved in cell motility (Verbost et al. 1987; Reddy et al. 1988) and we have demonstrated that inhibition of calmodulin also inhibits corneal epithelial cell migration. Inhibition by cadmium of calcium transport across the cell membrane and interference with calmodulin-mediated intracellular processing of calcium would be expected to inhibit cell migration. These effects of cadmium are therefore a logical explanation for the inhibition of corneal epithelial healing observed in this study but further experiments are required to confirm inhibition of calcium metabolism by cadmium in the sculpin cornea.

Mercury in its inorganic form, $HgCl_2$, or organic methyl mercury, CH_3HgCl (MeHg), also inhibited corneal epithelial cell migration in a dose-dependent manner (Figures 2B and 3A). At 10^{-7} M $HgCl_2$ was not toxic while at 10^{-5} M the healing rate was reduced to 2.1±0.42 mm^2/h compared to a control rate of 5.3±0.25 mm^2/hr. Control wounds closed by 7.5 h but healing stopped prior to wound closure after

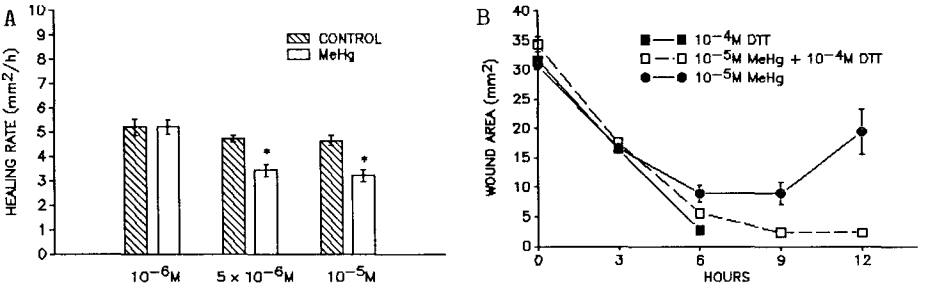

Figure 3. A. Effect of methyl mercury on corneal epithelial wound healing. * significantly different that paired control, $p \leq 0.05$. B. Dithiothreitol decreases the toxicity of methyl mercury. Mean±SE, n = 5.

9 h of exposure to 10^{-5} M $HgCl_2$. Video measurements of cell migration rate were not made in the presence of $HgCl_2$. Methyl mercury did not inhibit healing at 10^{-6} M, but the healing rate was reduced to 3.2±0.24 mm^2/h by 10^{-5} M MeHg compared to a control rate of 4.7±0.2 mm^2/h. Video recordings showed that 10^{-5} M MeHg reduced the cell migration rate to 0.26±0.01 mm/h. In contrast to the effects of $CdCl_2$ and $HgCl_2$, after 9-h MeHg caused cell sloughing with wounds enlarging by 12 h (Figure 3B). As is the case with cadmium, observations made by video microscopy showed that MeHg did not inhibit cell migration when added to the superfusate 3 h after the initiation of wound healing (data not shown).

It is apparent that $HgCl_2$ is toxic to corneal epithelium at a lower concentration than MeHg. While effects of inorganic and organic mercury on enzyme systems are similar, it has been reported that $HgCl_2$ has a greater direct effect on the cell membrane, causing disruption of its barrier function (Miura and Imura 1987).

Our finding that mercury is toxic to epithelial cells is in agreement with a previous report which shows that MeHg inhibits cell migration and causes degeneration of cultured neurons and astrocytes (Choi et al. 1981). Although Hg^{2+} is known to bind to calmodulin (Cheung 1984), it is more likely that the primary effect of mercury on corneal healing is via its high affinity for sulfhydryl (SH) groups, leading to inhibition of a wide variety of SH-dependent enzyme systems including ATPases and amino-acid transport systems (Miura and Imura 1987; Preston and Chen 1989). As pointed out by Miura and Imura (1987) the effects of mercury are so wide ranging that it is difficult to precisely identify its mechanism of toxicity. However, to test the SH hypothesis, wounded corneas were exposed to 10^{-5} M MeHg in the presence of 10^{-4} M DTT, a reducing agent that reverses the effects of mercury on thiol groups (Chen and Preston 1987). As shown in Figure 3B, DTT alone had no effect on corneal healing. The presence of DTT, however, significantly reduced the toxic effect of MeHg on wound healing and prevented the cell sloughing which occurred after 9-h exposure to MeHg alone. This confirms that inhibition of SH-dependent systems is involved in the inhibition of corneal epithelial healing by mercury.

Methyl mercury prevents mitosis by causing microtubule disassembly but has no effect on microfilaments in PtK_2 cells derived from kidney epithelium (Sager 1988). The phase of epithelial wound healing investigated in the present study involves little cell division and therefore MeHg inhibition of microtubules is probably not an important component of its toxic effects on corneal healing. Although the experiments using cytochalasins B and D clearly demonstrate the dependence of corneal epithelial cell migration on microfilaments, in preliminary experiments using fluorescent rhodamine phalloidin (unpublished data), we observed that 10^{-5} M MeHg has no apparent effect on distribution of actin filaments in sculpin corneal epithelium.

The results of these studies show that cadmium and mercury can have direct toxic effects on epithelial tissues of aquatic organisms which are exposed to these heavy metals. With respect to human development it is known that cadmium causes congenital malformations in mammals (Ferm 1971) and that methyl mercury causes developmental abnormalities of the nervous system related to inhibition of cell migration (Choi et al. 1978; Clarkson 1987). It appears that cadmium and mercury also have the potential to cause developmental anomalies related to epithelial cell migration.

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