CORNEAL EPITHELIAL CELL MIGRATION IN THE SCULPIN (MYOXOCEPHALUS OCTODECEMSPINOSUS): INTERACTION WITH METHYLMERCURY AND OTHER HEAVY METALS

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In a previous study we showed that the sculpin (Myoxocephalus octodecemspinosus) cornea in organ culture is a useful model for studies of epithelial cell migration (Ubels, et al., Bulletin MDIBL 27:100-101,1987-88). It was also demonstrated that this model, in which the cells migrate to cover a corneal abrasion at ten times the rate observed in mammals, is useful for studies of effects of toxic materials on cell migration. The heavy metals $CdCl_2$ and $HgCl_2$ inhibit cell migration at concentrations of 10^{-6}M or greater. In the current study we extended these investigations to include methyl mercuric chloride (MeHg). The time course of the effect of Hg and Cd was studied and corneas were stained for vinculin to determine whether assembly of this protein, which is essential for cell migration, is effected by MeHg.

Corneal epithelial wounds 6mm in diameter were made on sculpin corneas and the eyes were mounted in vitro for exposure to MeHg as previously described (Ubels et al., loc. cit.). The wounds were stained with fluorescein and photographed at three hour intervals. Wound areas and diameters were measured on the photographs. Corneas were also wounded and mounted for observation of cell migration by time lapse video microscopy. Corneas in various stages of the healing process were fixed with paraformaldehyde and vinculin was detected using antivinculin monoclonal antibody and FITC labeled IgG (Soong, Arch. Ophthalmol. 105:1129-1132, 1987). Corneas were observed and photographed with a fluorescence microscope.

The effects of MeHg on the rate of corneal epithelial wound closure are summarized in Figure 1. MeHg at 10^{-6}M was not toxic with all wounds closing by 9h. Wound closure rates for treated and control eyes did not differ and the cells migrated at 0.4 ± 0.03 mm/h. Wound healing rates were significantly different between controls and eyes treated with $5x10^{-6}$M MeHg; control and MeHg cells migrated at 0.37 and 0.29 mm/h, respectively. By 9h 80% of wounds exposed to MeHg remained open while all controls were healed. MeHg was highly toxic at 10^{-5}M. Healing rates were similar to those observed at $5x10^{-6}$M for the first 6-9h but after that point epithelium exposed to MeHg began to slough and the wounds enlarged. Initial healing rates are shown in Figure 1; cell migration rates were 0.36 ± 0.02 and 0.23 ± 0.02 mm/h, respectively. The toxicity of MeHg to the cornea can be reduced by dithiothreitol (Figure 2).

Cell migration was studied by videomicroscopy using corneas with 5mm diameter wounds. Under control conditions these wounds closed in about 6 h. This system was used to study the time course of the toxicity of $CdCl_2$, $HgCl_2$, and MeHg. The toxic effect of these metals has a latency of 2-3 hours as their introduction into the system 2h after the beginning of wound healing had no effect on overall cell migration rate or wound closure. Cell migration rates were significantly reduced when these toxins were introduced into the system at the beginning of the experiment.

Vinculin is part of a complex known as an adhesion plaque by which migrating cells are attached to basement membrane. It is concentrated along the migrating epithelial wound edge in the mammalian cornea (Zieske et al., J. Cell

Biol. 109:571-576, 1989). We were also able to detect synthesis of vinculin in the healing sculpin cornea; this is a significant finding since the monoclonal antibody used was raised against chicken vinculin and now has been shown to recognize fish as well as mammalian vinculin. This indicates that the protein is highly conserved among the vertebrates. Exposure to MeHg for up to 5h had no apparent effect on vinculin.

Our findings concerning MeHg have the following implications for human health; organic mercurials inhibit migration of fetal neural cells and astrocytes (Choi et al., Environ. Res. 24:61-74, 1981) and our data now show that MeHg can also inhibit migration of epithelial cells. These observations may explain some of the developmental abnormalities related to mercury poisoning. The data also have direct application to ophthalmology since some topical ophthalmic drugs are preserved with thimerosal. Some patients are intolerant of this compound and it is known that thimerosal can cause sloughing of the outer layer of corneal epithelial cells (Burstein and Klyce. Invest. Ophthalmol. Vis. Sci. 16:899-911,1977). Since a breakdown product of thimerosal is ethyl mercury, our data strongly suggest that drugs containing thimerosal should not be used in patients with corneal epithelial erosions. (This study was supported by NIEHS grant P30 ES03828)

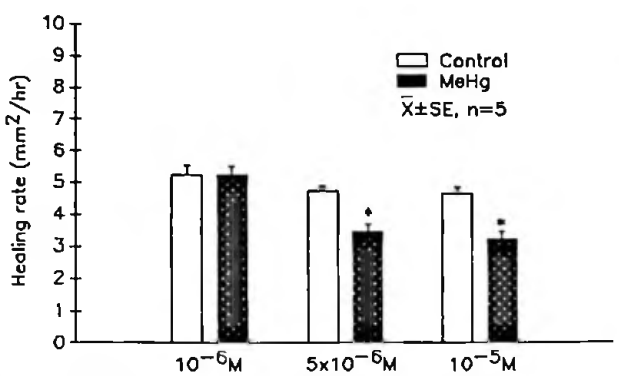

Figure 1. Inhibition of corneal healing by MeHg.

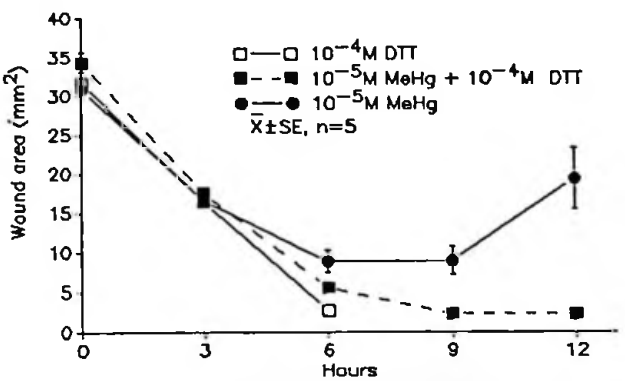

Figure 2. Dithiothreitol blocks MeHg toxicity.