THE EFFECT OF HEAVY METALS ON IN VITRO CORNEAL EPITHELIAL WOUND HEALING IN THE SCULPIN (Myoxocephalus octodecemspinosus)

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The corneal epithelium of the teleost is an essential barrier to ion and water fluxes across the cornea. Its integrity is essential to maintenance of corneal transparency and normal aqueous humor composition (Ubels and Edelhauser, Prog. Fish Cult. 49:219-224, 1987). We have previously reported that the corneal epithelium of the longhorn sculpin (Myoxocephalus octodecemspinosus) heals at an unusually rapid rate. The cells migrate to cover an abrasion at 3 mm^2/hr, in vivo (Ubels and Edelhauser, Curr. Eye Res. 2:613-619, 1982), as compared to the mammalian cornea which heals at about 0.8 to 1 mm^2/hr. In the present study an in vitro organ culture system was developed and used to study characteristics of corneal epithelial healing in the sculpin. The model was also used to study the effects of heavy metals on epithelial cell migration. Our rationale for this study was that the rapid healing rate of these corneas should allow us to maintain the eyes successfully in vitro and observe wound healing events in hours which normally occur over a period of 2-3 days in the mammalian cornea.

Sculpins were doubly pithed and a 7.5 mm diameter corneal epithelial abrasion was made on each cornea using a heptanol soaked filter paper disc. Both eyes were enucleated and one of each pair was mounted in a chamber and superfused with flowing sea water at 17°C. This chamber served as a control. The contralateral eye was mounted in a second chamber. A compound to be studied for effects on wound healing could be added to the water flowing through this chamber. This allowed healing rates to be compared on a paired basis. The compounds studied included cytochalasin B (a microfilament inhibitor), W-7 (a calmodulin inhibitor), tributyltin oxide (TBTO, the active ingredient in anti-fouling paints), $CdCl_2$, and $HgCl_2$ (heavy metals of environmental importance which may affect cell migration). Immediately after wounding and at 3 hr intervals up to 12 hr the corneal abrasions were stained with fluorescein and photographed. Wound areas were determined from the photos and healing rates (mm^2/hr) calculated by linear regression. Healing characteristics of the sculpin corneal epithelium were also studied by time lapsed video microscopy.

The mean initial wound area for all control eyes was 42.8 ± 0.69 mm^2. The mean healing rate for these eyes was 5.15 ± 0.13 mm^2/hr. Healing was complete within 10 hr in 64% of the eyes and wounds were present in only 11% of the eyes at 12 hr. On time lapsed video recordings we were able to observe the entire healing process to the moment of wound closure at 64 times actual speed and from these recordings we determined that the leading edge of the migrating epithelium moves at a linear rate of 0.5 mm/hr. By comparison a similar wound in the rabbit heals at 0.91 mm^2/hr with a linear rate of migration of 0.05 mm/hr. These wounds take 72 hr to close (Matsuda et al., Invest.Ophthalmol. Vis.Sci. 26:897-900, 1985).

As expected, cytochalasin B (5 ug/ml) completely inhibited wound healing, confirming that the cornea would respond to a known inhibitor of cell migration in our system. W7 (50 uM) caused a 42.5% decrease in healing rate indicating that corneal epithelial cell migration in the sculpin is calcium and calmodulin dependent.

TBTO, $CdCl_2$, and $HgCl_2$ all inhibited corneal wound healing, the magnitude of the inhibitory effect being concentration dependent (Table 1). The organotin was toxic at the environmentally relevant concentration of 10^{-9}M (U'ren, Marine Pollution Bull. 14:303-306, 1983). The mechanisms by which organotin, cadmium and mercury inhibit cell migration are unknown, however cadmium is known to inhibit calmodulin dependent processes (Scott et al., Exp. Cell Res. 156:191-197, 1985). The toxic effects of inorganic mercury suggest that organic mercury should also be studied in our system since methyl mercury is known to effect migration of neural cells (Choi et al., Environ. Res. 24:61-74, 1981).

The results of this study show that in vitro organ culture of sculpin eyes is a useful model for the study of effects of pollutants, such as heavy metals, on epithelial cell migration. This system will also be useful for studies of mechanisms of corneal wound healing since the eyes are easily maintained and the entire healing process can be studied during a period of only a few hours.

Table 1. Percent inhibition of corneal epithelial healing by heavy metals as compared to healing rates of paired control corneas.

	Concentration (M)				
	10^{-5}	10^{-6}	10^{-7}	10^{-9}	10^{-12}
TBTO	100(8)*	NT	88.8	27.7	0#
$CdCl_2$	56.3	23.7(9)	0#	NT	NT
$HgCl_2$	61.1	24.7	0#	NT	NT

NT=not tested
* n=5, except where indicated, (n)
# Healing rates not significantly different, paired t-test, $p>0.05$.

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