Zn^{2+} increases multidrug resistance-associated protein 2 (Mrp2) transport activity in killifish (*Fundulus heteroclitus*) renal proximal tubules

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One function of the vertebrate renal proximal tubule is the excretion into the urine of potentially toxic metabolites and foreign chemicals. This is accomplished through the action of numerous transport proteins located in the plasma membrane. Here we show that exposing killifish renal proximal tubules to $ZnCl_2$ rapidly and reversibly increases the activity of one such transporter, Mrp2, and that this increase is initiated through complex intracellular signaling.

Mrp2 is an ATP-driven efflux transporter that handles a wide range of metabolites, xenobiotics and xenobiotic metabolites. In renal proximal tubule, Mrp2 is localized on the luminal plasma membrane of the epithelial cells, where it can pump chemicals into the forming urine. We previously showed that exposing killifish renal tubules to $CdCl_2$ or $HgCl_2$ rapidly decreased Mrp2 transport activity, but then increased activity and transporter expression over many hours[1].

Zn is in the same group of the periodic table as Hg and Cd. It is an essential trace element and a cofactor for many enzymes. Zn deficiency affects about two billion people in developing countries and is associated with many diseases. To determine whether $ZnCl_2$ also reduced Mrp2 activity, we exposed isolated killifish renal tubules to 0.1-1 μM $ZnCl_2$ and measured transporter activity using a fluorescent substrate (Texas red), confocal imaging and image analysis [1]. The assay measured specific and concentrative accumulation of Texas red in the tubule lumens. To our surprise, $ZnCl_2$ exposure caused a rapid increase in Mrp2 transport activity; this effect was rapidly reversed when the $ZnCl_2$ was removed (Fig 1).

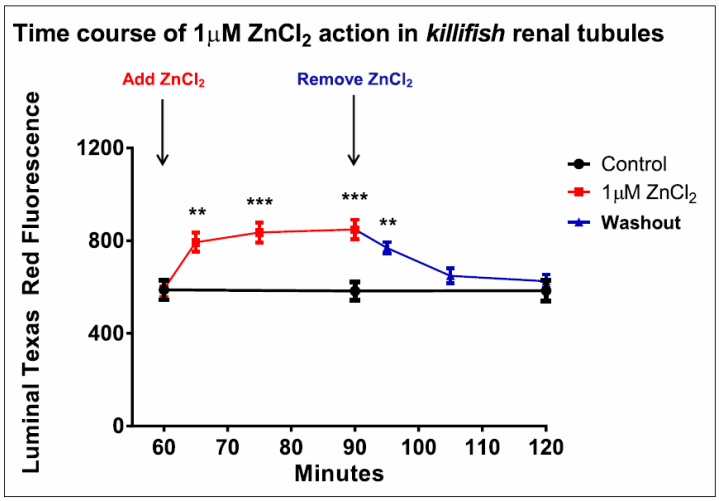

Figure 1. Time course of $ZnCl_2$ action in killifish renal tubules. Tubules were incubated to steady state with 2.5 μM Texas Red. At that time 1 μM $ZnCl_2$ was added to the medium. After 30 minutes tubules were transferred to $ZnCl_2$-free medium. Each point is the mean ± SE for 10-12 tubules from three fish. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Previous studies with $CdCl_2$ indicated that *reduced* Mrp2 transport activity was signaled through endothelin receptor (ET_B), NO-synthetase (NOS) and soluble guanylyl cyclase and protein kinase C (PKC)[1]. The present experiments with specific inhibitors of each of these receptors and enzymes showed that the same processes signaled $ZnCl_2$ induced *activation* of Mrp2 transport activity.

Recent experiments with rat brain capillaries and killifish renal tubules show that signalling through phosphoinositide-3-kinase (PI3K, protein kinase B), Akt and mTOR regulates basal P-glycoprotein activity (Ref. 2 and Cannon and Miller, *unpublished data*). In the present experiments with killifish renal tubules, we also found that sphingolipid signalling to PI3K, Akt and mTOR was also involved in the Zn-driven increase in Mrp2 activity. Based on the present findings we propose a complex signaling pathway through which $ZnCl_2$

increases Mrp2 activity in killifish renal tubules (Fig 2). At present, we have no data that speak to the fact that ET_B receptor/NOS/PKC signaling is common to a Zn-driven pathway that increases Mrp2 activity and a Cd-driven pathway that decreases Mrp2 activity. Our current thinking is that the pathways diverge at PKC, with each activating a different isoform, with one leading to sphingolipid and protein kinase signaling and the other leading elsewhere.

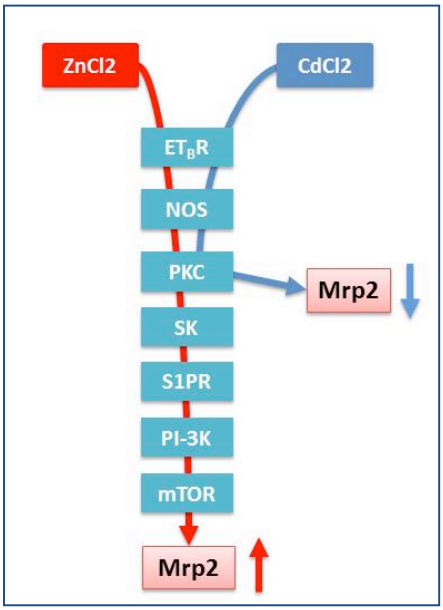

Figure 2. Signaling pathways through which $ZnCl_2$ and $CdCl_2$ regulate Mrp2 transport activity in killifish renal proximal tubules.

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2. **Terlouw SA, Graeff C, Smeets PH, Fricker G, Russel FG, Masereeuw R, Miller DS**. Short- and long-term influences of heavy metals on anionic drug efflux from renal proximal tubule. *J Pharmacol Exp Ther*. 301: 578-85, 2002.