Up-regulation of Mrp2 expression and transport activity by dexamethasone in killifish (*Fundulus heteroclitus*) renal proximal tubules

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The multidrug resistance-associated protein isoform 2 (Mrp2) is highly expressed at the luminal membrane of renal proximal tubule cells, where it excretes xenobiotics and metabolic wastes into the urinary space. Previous studies with isolated killifish tubules showed that Mrp2-mediated transport activity is rapidly reduced by endothelin-1 (ET-1) acting through an ET_B receptor, nitric oxide synthase (NOS), cyclicGMP and protein kinase C (PKC)[3,5,6]. This signaling system is also activated by a number of tubular nephrotoxicants[8]. In contrast, both ET-1 and nephrotoxicants increase Mrp2 transport activity and function 24 h after a transient exposure[7], suggesting transcriptional regulation. In mammalian liver, Mrp2 expression was shown to be transcriptionally regulated by ligand-activated nuclear receptors; the pregnane X receptor (PXR), constitutive androstane receptor (CAR) and farnesoid X receptor (FXR)[1]. Ligands for these receptors include both endogenous metabolites and xenobiotics. A recent screen of the *Fugu* genome shows that teleost fish also express analogous ligand-activated nuclear receptors, including, FXR and PXR, but not CAR[2]. In addition, using the cloned zebrafish receptor in a promoter assay, Moore et al.[4] identified several ligands for teleost PXR. The present experiments were designed to determine to what extent xenobiotics acting through these nuclear receptors can alter Mrp2 expression in killifish tubules.

Freshly isolated killifish kidney tubules were exposed for 3-24 h to xenobiotics known to be ligands for nuclear receptors. After exposure, tubules were incubated for 1 h in medium with 2 μM FL-MTX to assay Mrp2-mediated transport. Cellular and luminal FL-MTX accumulation was measured using confocal microscopy and quantitative image analysis (ImageJ software)[3]. In some experiments, tubules were immunostained with a Mrp2 specific antibody as described previously[3].

In initial screening experiments, the FXR ligand, chenodeoxycholic acid and zebrafish PXR ligands[4], dehydroisoandrosterone, n-propyl p-hydroxybenzoate, 15α-androstan 17β-ol, 5-β pregnane 3,20 dione, clotrimazole and pregnenolone-16-α-carbonitril, were tested at 1-50 μM. They had no effect on Mrp2 mediated transport. In contrast, the synthetic glucocorticoid, dexamethasone, potently increased Mrp2 mediated transport after 3 h exposure (Fig. 1). Furthermore, exposure to 1 μM dexamethasone for 3 hours increased expression of Mrp2 on the luminal plasma membrane of the tubular epithelial cells as determined by immunocytochemistry.

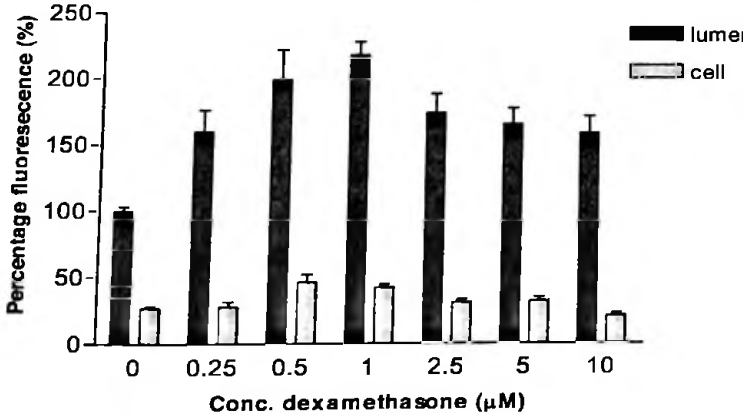

Figure 1. The effects of 3 hours incubation with dexamethasone on 2 μM FL-MTX transport. The fluorescence in the lumen and cell are shown as a percentage of the fluorescence intensity in the control lumen. All concentrations of dexamethasone tested significantly increased luminal FL-MTX transport ($p<0.01$; t-test). Means ± SEM are shown for 48-231 tubules.

Although Mrp2-mediated transport and expression increased after tubules were exposed to dexamethasone, it was not clear how this drug signaled the increases. Further experiments indicated that blocking the ET signaling pathway at NOS did not reduce dexamethsone's effects. Moreover, it was unlikely that dexamethasone acted through PXR, since a number of other PXR ligands were without effect (above). However, RU-486, a potent and specific blocker of the glucocorticoid receptor (GR) substantially attenuated the effects of dexamethasone on Mrp2-mediated transport (Fig. 2). Thus, Mrp2 expression in killifish renal proximal tubules appears to be transcriptionally regulated by GR, but not by PXR or FXR. This work was supported by the Netherlands Organization for Scientific Research (Zon-MW grant 902-21-227) and the MDIBL Center for Membrane Toxicity Studies.

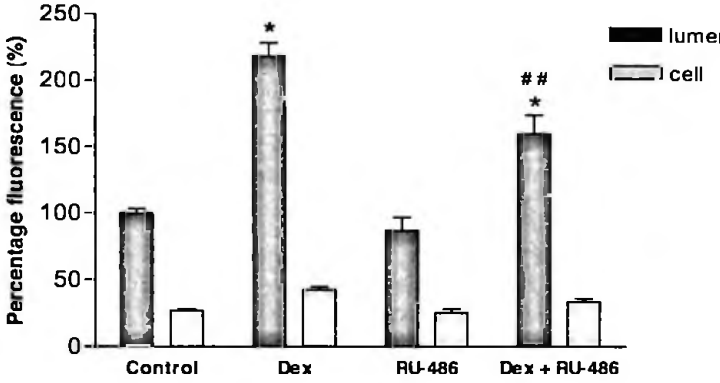

Figure 2. The effects of dexamethasone (dex) and RU-486 on FL-MTX transport. Tubules were incubated for 3 hours without (control) or with 1 μM dexamethasone, 1 μM RU-486 or both of these drugs. The fluorescence in the lumen and cell are shown as a percentage of the fluorescence intensity in the control lumen. Means ± SEM are shown for 49-231 tubules. * $p<0.001$ vs. control; ## $p<0.001$ vs. dex (one-way ANOVA).

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