Upregulation of multidrug resistance-associated protein (Mrp2) in renal proximal tubules from killifish, *Fundulus heteroclitus*

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Renal proximal tubules possess multiple plasma membrane transporters that drive active xenobiotic secretion into urine, including the multidrug resistance-associated protein isoform 2 (Mrp2). We showed previously that Mrp2 function is regulated by an endothelin-1 (ET-1) signaling pathway involving: ET_B receptor, nitric oxide, cGMP and protein kinase C[1,3,4]. Pathway activation decreases transport mediated by Mrp2. This pathway is also triggered by short-term exposure (30 min) to several nephrotoxicants, including the aminoglycoside antibiotic gentamicin[1,5]. We recently showed tubules exposed to ET-1 or to gentamicin and then transferred into drug-free medium recover Mrp2-mediated transport within 3-6 h and significantly increase transport after 24 h. This increase is abolished when the ET_B signaling pathway is blocked[2].

The present study was conducted to determine the molecular basis for the increase in Mrp2-mediated transport. We exposed isolated killifish renal proximal tubules to10 μM gentamicin or 10 nM ET-1 for 30 min and then transferred them to gentamicin- and ET-1-free medium for a 24 h recovery period. Tubules were then processed in PBS for immunostaining: fixation with 2% (v/v) formaldehyde/0.1% (v/v) glutaraldehyde; permeabilization with 1% (v/v) Triton X-100; 90 min exposure to primary antibody (k78 1:50 for Mrp2, or anti-Mrp4, 1:10); 60 min exposure to secondary antibody (Alexa488-labeled goat anti-rabbit IgG, 1:20). Antibody binding was detected with a Zeiss confocal laser scanning microscope and staining was quantified using ImageJ 1.30v (NIH, USA).

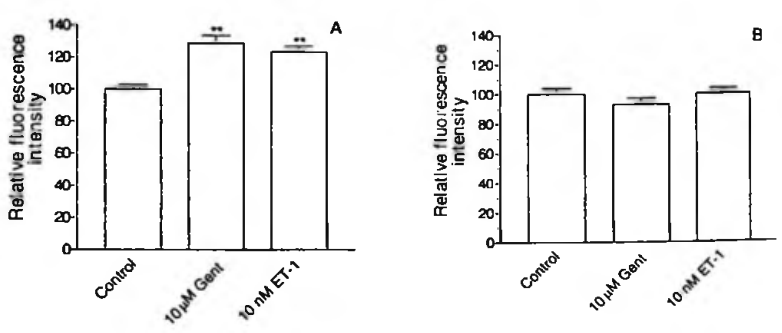

Fig. 1. Effects of short-term exposure to 10 μM gentamicin or 10 nM ET-1 followed by 24 h recovery on Mrp2 (A) and Mrp4 (B) immunostaining in killifish tubule luminal membranes. Data are given as mean ± SE for 31-108 tubules (**: significantly different than the control value; P<0.001)

In control tubules, both Mrp2 and Mrp4 showed a clear luminal (brush border) membrane localization. Tubules exposed to ET-1 or gentamicin and allowed to recover showed the same luminal staining pattern as controls. However, Mrp2 staining intensity increased significantly, 28% for gentamicin-exposed tubules and 23% for ET-1-exposed tubules (Fig. 1A). In contrast, Mrp4 immunostaining did not change (Fig. 1B). Additional experiments indicated that the increase in Mrp2 immunostaining was abolished when the nitric oxide synthase inhibitor L-NMMA (50 μM) was added to the recovery medium (not shown).

Thus, when renal proximal tubules were exposed to ET-1 or gentamicin and then allowed to recover for 24 h, Mrp2 expression in the luminal membrane of cells increased. This increase in expression parallels the previously observed increase in Mrp2 transport function[2]. As with the increase in Mrp2 transport function, the increase in expression seen after exposure and recovery was a result of signaling through the ET_B receptor, nitric oxide, cGMP and protein kinase C pathway. Since Mrp2 handles many potentially toxic xenobiotics and their metabolites, this may represent stimulation of a protective pathway following chemical insult. Supported in part by the MDIBL Center for Membrane Toxicity Studies and the Dutch Kidney Foundation.

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