Bisphenol-A modulates function of ABC transporters in killifish *(Fundulus heteroclitus)* renal proximal tubules

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Bisphenol-A (BPA) is a contaminant found in many marine and fresh water systems. We investigated the effect of BPA on the function of the excretory transport proteins in kidney tubules of the killifish *(Fundulus heteroclitus)*. BPA modulated transport properties of both efflux-proteins.

Bisphenol-A (BPA) is a main precursor in the production of plastic materials and a common water pollutant[1]. BPA is classified as an endocrine disruptor, functioning as an estrogen receptor agonist and as an androgen receptor antagonist. In fishes, BPA causes reproductive dysfunction, developmental deformation or feminization[1]. BPA has also been shown to be a substrate of ABC transporters that are ATP-driven drug efflux pumps: p-glycoprotein (P-gp, Abcb1), multidrug resistance-associated protein 2 (Mrp2, Abcc2) and breast cancer resistance protein (Bcrp, Abcg2) in human and rodent cell models[2]. In the present study we investigated the influence of BPA on transport function of Bcrp and Mrp2 in killifish renal proximal tubules.

Killifish were decapitated and kidney tubules were dissected as approved by the MDIBL IACUC. Tubules were then incubated at room temperature for 1 h in Tris-Forster buffer (TFB; 140 mM NaCl, 2.5 mM KCl, 1.5 mM $CaCl_2$, 1.0 mM $MgCl_2$, and 20 mM Tris at pH 8.0) with 0.2-1 μM BPA and 1 μM Texas Red (for Mrp2 assays) or 0.5-1 μM BPA and 1 μM Mitoxantrone (for Bcrp assays). Kidney tubules incubated for 1 h with Mitoxantrone or Texas Red without BPA served as negative controls. Tubules were imaged using a Zeiss meta 510 confocal laser scanning microscope (Karl Zeiss GmbH, Jena, Germany). Luminal fluorescence intensities were measured from saved images using Image J software (National Institutes of Health, Bethesda, Maryland, USA). These provide a measure of Mrp2 (Texas red) and Bcrp (Mitoxantrone) activity. For each experiment kidneys of 3-6 fish were prepared. Images of 10 to 15 tubules per treatment were analysed. One-Way ANOVA was used to support statistical significance.

BPA reduced Mrp2-mediated transport of Texas Red in a concentration-dependent manner. With 0.2 μM BPA luminally directed transport was reduced 43±6 %, and with 1 μM BPA transport to the lumen was diminished by 62±4%. This indicates a strong inhibitory effect of BPA on Mrp2 transport function. In contrast, BPA increased Bcrp-mediated, luminal Mitoxantrone accumulation by 23±4% and 35±7% for 0.5 and 1.0 μM BPA, respectively. Cellular fluorescence remained unchanged compared to control levels in all experiments. Data were calculated from two (Bcrp) or three (Mrp2) independent experiments.

The mechanisms by which BPA reduces Mrp2-mediated transport but increases Bcrp-mediated transport are unclear. It is possible that BPA inhibits transport on Mrp2, but increases Bcrp expression and thus its transport activity. Future studies will focus on an understanding of the mechanisms involved and on the in vivo consequences of alter xenobiotic excretion in fish kidney.

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1. **Flint S, Markle T, Thompson S, Wallace E.** Bisphenol-A exposure, effects, and policy: a wildlife perspective. *J Environ Manage* 104: 19–34, 2012.
2. **Mazur CS, Marchitti SA, Dimova M, Kenneke JF, Lumen A, Fisher J.** Human and rat ABC transporter efflux of bisphenol-A and bisphenol-A glucuronide: interspecies comparison and implications for pharmacokinetic assessment. *Toxicol Sci* 128: 317–325, 2012.