Resveratrol interacts with multiple transporters in killifish, *Fundulus heteroclitus*, renal proximal tubules

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Resveratrol (trans-3,5,4′-trihydroxystilbene) is a constituent of grapes, berries and peanuts with cardioprotective and anticancer properties[1,7]. Although there is some evidence that the compound can enter cells by a mediated pathway, nothing is known about the transporters involved[2]. To determine whether resveratrol interacts with xenobiotic transporters, we used killifish renal proximal tubules as a test system to measure its effects on the transport of three fluorescent compounds: NBD-cyclosporine A (NBD-CSA), a substrate for p-glycoprotein, fluorescein-methotrexate (FL-MTX), a substrate for multidrug resistance-associated protein isoform 2 (MRP2), fluorescein (FL), a substrate for organic anion transporter 1 and 3, (OAT1/3).

Renal proximal tubules were isolated from killifish and maintained in a teleost marine saline medium (in mM: 140 NaCl, 2.5 KCl, 1.5 $CaCl_2$, 1.0 $MgCl_2$, 20 Tris, pH 8.0). For experiments, tubules were transferred to confocal chambers containing medium with fluorescent compound without (control) or with 1-50 μM resveratrol. After 60 min, the steady state distribution (epithelial cells and tubular lumen) of the fluorescent compound was measured using confocal microscopy and quantitative image analysis as described previously[3].

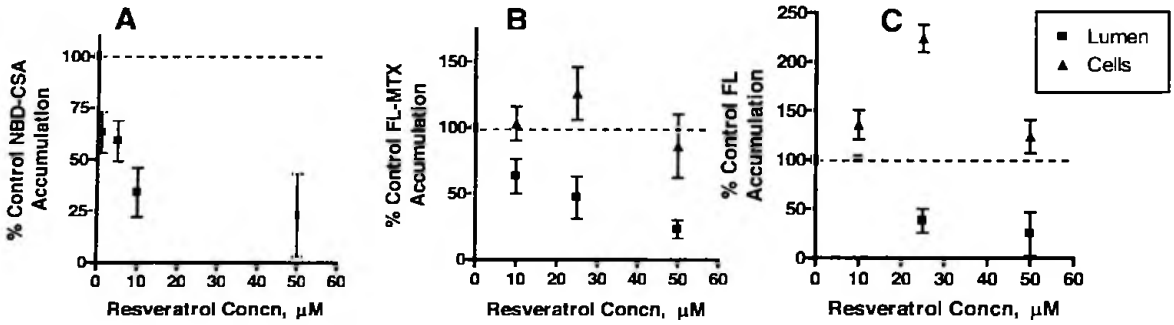

Figure 1. Effects of resveratrol on transport of fluorescent xenobiotics in killifish renal proximal tubules. Each point represents the mean value for data from 12-35 tubules from 2-6 fish; variability is shown as SE bars. The broken lines indicate the control levels.

Initial experiments indicated that resveratrol concentrations as high as 50 μM caused no obvious changes in tubular morphology. In spite of this, resveratrol did reduce the transepithelial transport of all three fluorescent compounds (Fig. 1), indicating interactions with all of the transporters involved. Resveratrol was a potent inhibitor of luminal NBD-CSA accumulation, with an IC_{50} value (concentration causing 50% inhibition) of about 5 μM (Fig. 1A). In previous experiments carried out under similar conditions, potent and specific p-glycoprotein inhibitors, e.g., PSC833, exhibit submicromolar IC_{50} values[4]. In this assay, the effectiveness of resveratrol appeared to be comparable to some of the Ca-channel blockers, which exhibit IC_{50} values in the 5-10 μM range[4]. Resveratrol was

clearly less effective as an inhibitor of organic anion (FL-MTX and FL) transport. With FL-MTX, resveratrol did not alter cellular accumulation, but significantly reduced luminal accumulation at all three concentrations tested ($P<0.01$; t-test: Fig. 1B). The IC_{50} was about 25 μM. This is substantially higher than that found for leukotriene C_4 and MK571, potent inhibitors of MRP-mediated transport[4].

The pattern of effects for FL transport was more complex (Fig. 1C). FL transport across the tubular epithelium involves two concentrative steps[6]. The first step is mediated by an OAT and the second step by an as yet unidentified transporter. Cellular accumulation of FL was slightly stimulated by 10 μM resveratrol and more than doubled by 25 μM resveratrol; cellular accumulation returned to control levels with 50 μM resveratrol. Luminal accumulation was not reduced at 10 μM resveratrol, but was significantly reduced ($P<0.01$; t-test) at 25 and 50 μM. For luminal FL accumulation the IC_{50} was about 25 μM. Since no decrease in cellular accumulation of FL was observed it is unlikely that resveratrol interacts with the basolateral OAT present in these tubules. This transporter mediates Na-dependent, organic anion uptake and its ability to function is dependent on cellular metabolism[6]. Thus, the lack of reduction in cellular FL accumulation suggest that resveratrol did not reduce NBD-CSA and FL-MTX transport by inhibiting metabolism. The observed increase in cellular FL accumulation may be secondary to blocked luminal efflux, a phenomenon seen previously[5].

The present results for a comparative model system indicate that resveratrol interacts with high potency with p-glycoprotein and with moderate potency with MRP2. It does not appear to interact with the basolateral OAT present in the tubules, but is a moderate inhibitor of cell to lumen organic anion transport. Obviously, inhibition of transport does not necessarily mean that the inhibitor is also transported. Thus, additional studies will be needed to determine which transporters influence the cellular accumulation of resveratrol and its distribution within the body. Supported in part by the Maren Foundation and the MDIBL Center for Membrane Toxicity Studies.

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