p-GLYCOPROTEIN-MEDIATED SECRETION OF A FLUORESCENT RAPAMYCIN DERIVATIVE BY KILLIFISH RENAL PROXIMAL TUBULES

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Rapamycin is a potent, macrocyclic immunosuppressive drug that blocks transduction of cytokine signals needed for proliferation and maturation of T cells (Sehgal et al, Med. Res. Rev. 14:1, 1994). The therapeutic potency of the drug is up to 100 times greater than that of cyclosporin A (CSA) and there is evidence that the renal side effects of rapamycin, such as tubular atrophy and interstitial fibrosis, are less severe than for CSA (Thliveris et al., Histol. Hisopathol. 10:417, 1995). At present, it is not clear whether specific drug transport systems handle rapamycin or whether it distributes through the body by simple diffusion. However, available data suggest that rapamycin may be capable of functioning as an multidrug resistance (MDR) reversing agent by interacting with p-glycoprotein (the MDR transporter; Arceci et al., Blood 80:1528, 1992). These findings document an interaction between rapamycin and p-glycoprotein, but they do not address the question of whether the drug is a substrate for transport. In the present study we used confocal microscopy and digital image analysis to study the transport of a fluorescent rapamycin derivative, (4-nitrobenzofurazan-7-yl)-rapamycin (NBD-rapamycin), in intact renal proximal tubules from a teleost fish.

Renal tubular masses were isolated in a marine teleost saline (containing, in mM:140 NaCl, 2.5 KCl, 1.5 $CaCl_2$, 1.0 $MgCl_2$ and 20 tris(hydroxymethyl)-amino methane, at pH 8.0). Individual killifish proximal tubules were dissected free and transferred to a Teflon chamber (Bionique) containing 1 ml of marine teleost saline with 0.5-1 μM NBD-rapamycin and added effectors. The chamber floor was a 4x4 cm glass cover slip to which the tubules adhered lightly and through which the tissue could be viewed by means of a Noran Confocal Microscope with a Nikon 40x Fluor oil immersion objective (NA 1.4). Illumination was provided by an Ar laser at 488 nm. A 510 nm dichroic filter and a 515 nm long pass emission filter were employed. To obtain an image, dye-loaded tubules in the chamber were viewed under transmitted light and a single proximal tubule with well defined lumen and undamaged epithelium was selected; the plane of focus was adjusted to cut through the center of the tubular lumen. In confocal fluorescence mode, 128 video frames were averaged. The confocal image (512x512x8 bits) was viewed on a high resolution monitor and saved to optical disk. Fluorescence intensities were measured from stored images using an Apple Power Macintosh 7100 computer and NIH Image version 1.58 software as described previously (Miller, Am. J. Physiol. 269:F370, 1995).

Tubules incubated in medium containing 1 μM NBD-rapamycin exhibited rapid increases in fluorescence intensity in both the cellular and lumenal compartments (not shown). At steady state (60 min), luminal fluorescence exceeded cellular fluorescence. In experiments with 94 control tubules from 8 fish the lumen-to-cell fluorescence ratio averaged 3.3 (range 1.8-4.1). Addition of 1 mM KCN to the medium had no effect on cellular fluorescence, but reduced luminal fluorescence to the level of the cells. Table 1 shows the effects of various agents on the transport of 0.5-1.0 μM NBD-rapamycin by killifish tubules. None of the compounds tested altered cellular fluorescence. The parent compound, rapamycin, was a potent inhibitor of luminal accumulation of NBD-rapamycin. Other inhibitors of luminal accumulation included, cyclosporin A (CSA), PSC-833, FK506 and verapamil, some of which (PSC-833 and FK506) were effective at submicromolar

Table 1. Effects of transport inhibitors on NBD-rapamycin accumulation.

Treatment	n	Fluorescence Intensity	
		Cell	Lumen
Control (0.5 μM)	12	25 ± 3	79 ± 8
1 μM Rapamycin	7	31 ± 3	29 ± 4**
5 μM Rapamycin	9	34 ± 7	15 ± 3**
5 μM Cyclosporin A	11	25 ± 4	14 ± 2**
Control (1.0 μM)	9	16 ± 2	50 ± 5
1 mM p-Aminohippurate	5	19 ± 3	53 ± 6
1 mM Tetraethylammonium	7	17 ± 1	52 ± 2
0.5 μM Leukotriene C_4	6	14 ± 2	47 ± 5
100 μM Verapamil	7	18 ± 3	11 ± 1**
Control (1.0 μM)	7	10 ± 1	31 ± 4
0.1 μM FK506	7	10 ± 1	16 ± 3*
1.0 μM FK506	7	12 ± 2	8 ± 2**
Control (1.0 μM)	8	11 ± 1	37 ± 6
0.1 μM PSC-833	9	13 ± 1	25 ± 3
0.5 μM PSC-833	7	12 ± 2	16 ± 2**
1.0 μM PSC-833	7	15 ± 2	11 ± 1**

Tubules were incubated for 60 min in medium containing NBD-rapamycin without (control) and with the indicated chemicals. The substrate concentration used for each experiment is in parentheses. NBD-rapamycin and many of the drugs used were added to the medium as stock solutions in dimethyl sulfoxide (DMSO; final DMSO concentration 0.05-1%). Preliminary experiments showed that DMSO in this concentration range has no effect on drug transport in killifish tubules. Data are given as mean ± SE; n is the number of tubules.Statistical comparisons: * significantly lower than controls, $P<0.05$; ** significantly lower than controls, $P<0.01$.

concentrations. All of these drugs are substrates for or modifiers of p-glycoprotein. In contrast, inhibitors of the other xenobiotic transport systems present in killifish proximal tubule, p-aminohippurate for the classical organic anion system, tetraethylammonium for the organic cation system and leukotriene C_4 for the Na-independent system for large organic anions (Pritchard and Miller, Physiol. Rev. 73:765, 1993; Masereeuw et al., Am. J. Physiol., in press), used at concentrations that should have blocked transport on those systems, were without effect (Table 1). In addition, HPLC analyses of tubule extracts and incubation media showed that NBD-rapamycin was not degraded during transport experiments. Thus, NBD-rapamycin entered killifish proximal tubule cells by simple diffusion. It was, however, transported from cell to tubular lumen by a process that was saturable (data not shown), concentrative, dependent on cellular metabolism and inhibited by rapamycin, CSA and other p-glycoprotein substrates. It appears that NBD-rapamycin and possibly the parent compound, rapamycin, are actively secreted into the tubular lumen of proximal tubules by p-glycoprotein. Supported in part by a grant from the Deutsche Forschungsgemeinschaft to G.F.