Scott D. Campbell
Dr. sc. hum.

The Role of Hepatic Transporters in the Uptake and Efflux of Porphyrins

Geboren am 16. Januar 1962 in Norwalk, CT, USA
Diplom (Master of Science) der Fachrichtung "Cellular and Molecular Biology",
Quinnipiac University, CT, USA

Promotionsfach: Biochemie
Doktorvater: Prof. Dr. med. Dietrich Keppler

Porphyrins are cyclic tetrapyrroles and represent an important class of photoreactive compounds that function as cofactors of cellular proteins such as cytochromes, hemoglobin, and myoglobin. Porphyrin homeostasis is tightly controlled and an impairment of this leads to several photosensitivity and neurological disorders.

While it is traditionally thought that porphyrins cross cell membranes by passive diffusion, many studies have shown that minor changes in the length, charge or number of attached side chains can strongly alter the compounds' clearance and tissue distribution. Several groups have shown that porphyrins are substrates for the efflux transporter ABCG2 (Breast Cancer Resistance Protein, BCRP), however, no studies have been reported examining the role uptake transporters play in the hepatic clearance of these compounds.

The aim of this work was: (1) to functionally characterize the interaction of porphyrins with transporters expressed on both the basolateral and apical surface of hepatocytes, (2) to identify which transporters are involved in porphyrin clearance, and (3) to determine whether a correlation exists between compounds that inhibit these cloned transporters and compounds that have been clinically shown to cause increases in the systemic concentration of porphyrins.

At the beginning of this study little was known of the structural features of the porphyrins important for interactions with the human hepatic uptake transporter OATP1B1 (organic anion transporting polypeptide 1B1). In order to rank the affinity of various porphyrins for OATP1B1, compounds were evaluated for their ability to inhibit the OATP1B1-

mediated uptake of the standard substrate estradiol 17β-D-glucuronide. Of the linear tetrapyrroles tested, ditaurine-conjugated bilirubin was the most potent inhibitor of uptake with an IC_{50} of 5 nM, while substitution of the taurine side chains with methyl ester groups eliminated the ability of bilirubin to inhibit uptake. Hematoporphyrin (HP), a cyclic porphyrin with 2 hydroxyethyl and 2 propionic groups attached to the pyrrole ring, had an IC_{50} of 60 nM, while porphyrins lacking charged side chains, such as etioporphyrin and phthalocyanine, did not inhibit OATP1B1-mediated uptake up to 100 μM. Uroporphyrin which is cleared renally did not inhibit OATP1B1-mediated uptake, while bilirubin and protoporphyrin, which accumulate in hepatocytes, did.

To further characterize the ability of porphyrins to interact with OATP transporters expressed in human hepatocytes, inhibition studies were conducted for OATP1B1-, OATP1B3-, and OATP2B1-mediated uptake of labeled bromosulfophthalein (BSP). For all three transporters, HP was the most potent inhibitor with K_i values ranging between 0.7 and 3.2 μM, however inhibition was not detected in a HP derivative in which the propionic side chains were replaced with methyl ester groups.

Direct measurements of transporter-mediated porphyrin transport were performed using MDCK cells stably expressing either an OATP or an efflux transporter (Multidrug-resistance protein (MRP2) or ABCG2) or a combination of uptake and efflux transporters. Of the porphyrins tested the highest rate of OATP/MRP2-mediated vectorial transport was observed for HP. The ratio of apparent permeability (P_{app}) for basolateral-to-apical over apical-to-basolateral transport was at least 10-fold higher than that observed with either the MDCK parental or OATP or MRP2 single- transfected cell lines. The highest rate of vectorial transport was observed for the OATP2B1/MRP2-expressing cell line with a ratio of 22.9±1.6, while the OATP1B1/MRP2- and OATP1B3/MRP2-expressing cell lines had ratios of 13.7±1.4 and 11.5±2.2, respectively. Vectorial transport of porphyrins was also evaluated using OATP/ABCG2-expressing cells. As with the MRP2-expressing cells, HP and Chlorin e6 demonstrated the highest rate of vectorial transport with P_{app} ratios between 3- and 6-fold higher in the OATP/ABCG2-expressing cells than in the wild-type MDCK cells. For both the OATP/MRP2- and OATP/ABCG2-expressing cells, porphyrin transport was inhibited up to 90% in the presence of compounds clinically linked to porphyrias.

In conclusion, this work demonstrates the obligatory role of uptake and efflux transporters in the hepatic clearance of porphyrins and lays the foundation for clinical studies to explore the relationship between inhibition of transporters and drug-induced pseudoporphyria.