Fluorescent Bile Salt Derivatives for the Investigation of the Canalicular Lipid Transporter System

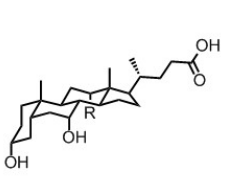
M. Leuenberger[1,3], S. Bernhard[2], B. Stieger[2], M. Lochner[1,3]*

[1]Department of Chemistry and Biochemistry, [2]Department of Clinical Pharmacology and Toxicology, [3]Institute of Biochemistry and Molecular Medicine

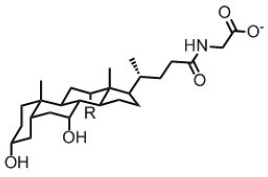
The liver is the biggest gland and a vital organ for animals and humans. Hepatocytes are complex cells responsible for the metabolism and filtration of xenobiotics and toxic compounds from the body. The correct function of these cells is crucial for a healthy organism. One of their major functions is the formation of the bile. The canalicular bile formation implicates the concomitant interplay of a series of ABC-transporters, i.e. BSEP (Bile salts export pump), MDR3 (or ABCB4) and ABCG5/G8. This process regulates the quantity of all ingredients and ensures the formation of a healthy bile. The disruption of the equilibrium between cholesterol, phospholipids and bile salts can lead to cholestatic liver injury. For instance, several drugs interact with this system, although the exact mechanism remains uncertain.

Here, we present the preparation of fluorescent bile salt derivatives in order to investigate the transport of the bile salts from the blood into the hepatocytes and then their secretion into the *canaliculi*. Three different fluorescent dyes were coupled to the side chains of cholic acid (CA) and Chenodeoxycholic acid (CDCA) to mimic known transport substrate Glycocholate (GC) and Glycochenodeoxycholate (GCDC). Nitrobenzofurazan (NBD), dansyl and a coumarin dye (Pacific Blue) were selected to investigate the impact of these different dyes on transport behaviour. Transport of the synthetic fluorescent bile salts was assessed in CHO cells expressing NTCP (Na+-taurocholate co-transporting polypeptide), organic anion-transporting polypeptides OATP1B1, OATP1B3 or OATP2B1, as well as in Sf9 cell vesicles expressing BSEP.

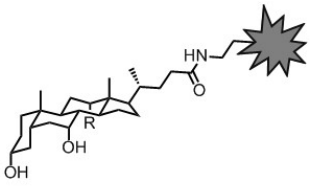
Our data show that the probes are transported with different selectivity with respect to the various transporters and that subtle structural changes can have a significant impact on transport behaviour. As such, our florescent bile salt probes might be promising tools for the selective examination of the individual transport pathways.



R = OH; **Cholic acid** (**CA**)
R = H; **Chenodeoxycholic acid** (**CDCA**)



R = OH; **Glycocholate** (**GC**)
R = H; **Glycochenodeoxycholate** (**GCDC**)



Fluorescent bile salts