The familial intrahepatic cholestasis type 1 protein: a P-type ATPase influencing bile acid transporters

M.J. Harris[1,2] and I.M. Arias[2], [1]ANZAC Research Institute, University of Sydney, Concord RG Hospital, Concord, NSW 2139 and [2]Tufts University School of Medicine, Boston MA, USA.
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Progressive familial intrahepatic cholestasis type 1 (PFIC1) and benign recurrent intrahepatic cholestasis (BRIC) result from mutations in the familial intrahepatic cholestasis gene (FIC1). FIC1 is a member of the type IV P-type ATPase subfamily, which function as aminophospholipid translocases. Since the phenotype of these diseases manifests as impaired bile flow and considering that FIC1 is localised to the canalicular membrane in hepatocytes, we investigated whether FIC1 could transport bile acids and/or influence the apical bile acid transporters, either the bile salt export pump (BSEP) or the intestinal apical sodium dependent bile acid transporter (ASBT).

Method: Apical efflux assay: MDCK II cells which stably express Na^+/taurocholate co-transporting polypeptide (NTCP) formed polarised monolayers when grown on Transwell filters and were transfected with FIC1 and/or BSEP. Two days after transfection, the basal medium was replaced with uptake buffer containing ^{3}H-taurocholate (TC) and the cells were incubated at 37°C for 1 hour. TC efflux was determined by measurement of radioactivity in the apical medium. Apical uptake assays: MDCK II cells were transfected with FIC1, FIC1 mutants and/or ASBT. Two days after transfection, the apical media was replaced with uptake buffer containing ^{3}H-TC and incubated at 37°C for 1 hour. Uptake of ^{3}H-TC by ASBT was determined by measurement of intracellular radioactivity. In all studies, transfection with β-gal was used as a control and western blotting of membrane preparations confirmed expression of each relevant protein.

Results: Apical efflux: ^{3}H-TC apical efflux in BSEP transfected cells was 2 fold higher than in non-transfected MDCKII-NTCP cells ($p<0.05$) and was unaffected by co-transfection of FIC1. In addition, FIC1 expression had no effect on ^{3}H-TC efflux in cells which were not transfected with BSEP. Apical uptake: ^{3}H-TC uptake in ASBT expressing cells was 10 fold higher than in β-gal or FIC1 transfected cells, and was unaffected by transfection of cells with both ASBT and FIC1, or ASBT and FIC1 mutants.

Summary: FIC1 did not transport taurocholate across the apical membrane of MDCK II cells. Expression of FIC1 or FIC1 mutants did not affect BSEP or ASBT function. These results suggest that FIC1 affects hepatic bile secretion and/or intestinal bile acid absorption by indirect mechanisms that are currently unknown. An alternative hypothesis is that FIC1 effects the trafficking of bile acid transporters to the apical membrane via its aminophospholipid translocase activity. This “flippase” function is required for the budding of vesicles from organelles such as the golgi, endosomes and the plasma membrane. These mechanisms are being investigated by siRNA knockout experiments.