01C09-1

BASOLATERAL EFFLUX OF FOLATES AND THEIR ANALOGS AND GLUCURONIDE CONJUGATES IS MEDIATED BY MRP3 IN THE SMALL INTESTINE

Hirohiko Kusuhara, Yoshiaki Kitamura, and Yutaka Sugiyama

1Graduate School of Pharmaceutical Sciences, The University of Tokyo, Tokyo, Japan; 2Discovery Research Laboratories, Kyorin Pharmaceutical Co. Ltd., Tochigi, Japan

Multidrug resistance associated protein 3 (Mrp3/Abcc3) is an ABC transporter which accepts a variety of compounds, such as bile acids, folates, methotrexate and glucuronide conjugates of xenobiotics. In the intestine, Mrp3 is expressed most abundantly in the colon followed by the duodenum, jejunum and ileum where it is localized on the basolateral membrane. The present study investigated the role of Mrp3 in the intestinal absorption. Mucosal-to-serosal transport was determined in vitro using everted intestinal sacs. The basolateral efflux rate of the intracellularly formed glucuronide conjugate of 4-methylumbelliferone was significantly reduced in Abcc3−/− mice throughout the small intestine compared with wild-type mice, although a similar amount of the glucuronide conjugate was produced during the experiment in wild-type and Abcc3−/− mice. In addition, the mucosal-to-serosal transport rates of folic acid, leucovorin and methotrexate were significantly reduced in everted sacs from the duodenum of Abcc3−/− mice compared with wild-type mice. The effect of Mrp3 impairment was weaker for leucovorin than folic acid and methotrexate. In particular, the intrinsic efflux clearance of methotrexate across the basolateral membrane in Abcc3−/− mice was 23% of the corresponding values in wild-type mice. However, unlike the glucuronide conjugate, such alteration was observed only in the duodenum, and not in the jejunum and ileum. This may be accounted for by the heterogeneous distribution of uptake transporters, such as PCFT and RFC, along the intestine. In conclusion, Mrp3 facilitates the intestinal absorption of methotrexate and folates in the duodenum in cooperation with uptake transporters.

01C09-2

PPAR ALPHA-MEDIATED TRANSACTIVATION OF HUMAN NPC1L1

Tappei Takada, Yuki Iwayamagi and Hiroshi Suzuki

Department of Pharmacy, The University of Tokyo Hospital, Tokyo 113-8655, Japan

Niemann-Pick C1-like 1 (NPC1L1) is recognized as an intestinal cholesterol importer and as a pharmacological target of ezetimibe. Recently, it was demonstrated that the sterol responsive element binding protein 2 (SREBP2) is responsible for the cholesterol-dependent transcription of NPC1L1 and the hepatocyte nuclear factor 4α (HNF4α) is a crucial modulator of the regulation. In the present study, we focused on the involvement of the peroxisome proliferator-activated receptor α (PPARα), which works as a key modulator of lipid homeostasis, in the transcriptional regulation of human NPC1L1. Reporter gene assays with the 5′-flanking region of human NPC1L1 gene showed a PPARα-mediated transactivation. Detailed analyses using the deletion- and mutated-promoter constructs revealed the presence of a functional PPARα-response element (PPRE) upstream of human NPC1L1 gene, the direct binding of PPARα and retinoid X receptor α (RXRα) to which was confirmed by EMSAs. Moreover, PPARα-specific knockdown led to a significant decrease in the endogenous expression of NPC1L1 mRNA in human-derived HepG2 cells. Furthermore, cotransfection of PPARγ coactivator 1α (PGC1α), a common coactivator of nuclear receptors, stimulated SREBP2/HNF4α- and PPARα/RXRα-mediated activation of human NPC1L1 promoter. Collectively, we found that PPARα positively regulates the expression of human NPC1L1 via a direct binding to a PPRE upstream of the gene and PGC1α stimulates the transactivation of human NPC1L1. It is possible that the known diurnal oscillation in the intestinal cholesterol absorption may be accounted for by a circadian variation of NPC1L1 expression mediated by these transcriptional factors.