EFFECT OF TICLOPIDINE ON MDR3/ABCB4-MEDIATED BILIARY SECRETION OF PHOSPHOLIPIDS
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[Purpose] Multidrug resistance 3 P-glycoprotein (MDR3/ABCB4) and bile salt export pump (BSEP/ABCB11) mediate biliary secretion of phospholipids and bile acids, respectively, and their genetic disruptions cause severe cholestasis. Based on a hypothesis that some cholestatic drugs may inhibit the function of these transporters, we previously investigated the involvement of MDR3 inhibition in itraconazole-induced cholestasis. In this study, we focused on ticlopidine, which was found as another MDR3 inhibitor. [Methods] Thirty cholestatic drugs were checked with their effects on the efflux of [14C]phosphatidylcholine from MDR3-expressing LLC-PK1 cells. Among them, ticlopidine was found as one of MDR3 inhibitors and we further examined its effect on the transcellular transport of [3H]taurocholate in BSEP and Na+/taurocholate cotransporting polypeptide (NTCP/SLC10A1)-coexpressing cells. To characterize the effect of ticlopidine in vivo, ticlopidine was administered to SD rats and the bile composition was analyzed. [Results and Discussion] Ticlopidine showed a significant inhibitory effect on the function of MDR3, but not on that of BSEP, and biliary phospholipids rather than bile acids were significantly decreased in ticlopidine-administered rats. In addition, the administration of ticlopidine stimulated the biliary secretion of glutathione, an endogenous antioxidant in hepatocytes, and decreased glutathione concentrations in the liver, which can also potentially contribute to the development of liver injury. [Conclusions] These results suggested that the inhibition of MDR3-mediated biliary secretion of phospholipids may be involved in cholestasis induced by ticlopidine. Our approach may be useful for evaluating cholestatic potential of clinically-used drugs and drug candidates.

CALPAIN-MEDIATED CLEAVAGE NEGATIVELY REGULATES THE EXPRESSION LEVEL OF ABCG1
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[Purpose] ABCG1, a half-transporter belonging to the ATP-binding cassette subfamily G, has been identified as an important molecule against atherosclerosis, which is implicated in the export of cholesterol from macrophages to HDL, and inhibits the development of the atherosclerotic lesion areas in high fat-fed mice. Much less is known about the regulatory mechanism of ABCG1, while its physiological importance is becoming clearer. In the present study, we investigated the underlying mechanism of ABCG1 degradation, specifically focusing on calpain, a family of calcium-dependent proteases, because ABCG1 has a potential consensus sequence for calpain-mediated cleavage. [Methods] HEK293 cells expressing human ABCG1 (ABCG1-HEK cells) and mouse peritoneal macrophages were used to investigate the involvement of calpain-mediated cleavage in ABCG1. [Results and Discussion] Purified μ-calpain cleaved ABCG1 in crude membrane fractions prepared from ABCG1-HEK cells. In ABCG1-HEK cells, calpeptin treatment, a calpain inhibitor, significantly inhibited ABCG1 degradation and thereby increased the expression and cholesterol efflux function of ABCG1. Studies using a biotinylation technique demonstrated significant prolongation of the half-life of ABCG1 on the cell surface with calpeptin treatment as well as greater ABCG1 induction with calpeptin treatment in cell surface fractions than that in whole cell lysates, suggesting calpain cleaves cell surface-resident ABCG1. In mouse peritoneal macrophages, calpeptin treatment also inhibited ABCG1 degradation and enhanced ABCG1 expression. [Conclusions] Calpain promotes ABCG1 degradation by cleavage of ABCG1 on plasma membrane and thereby negatively regulates the expression and cholesterol efflux function of ABCG1.