01C09-3

IN VITRO CHARACTERIZATION OF THE CLUSTERED GENETIC VARIANTS OF NPC1L1
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Niemann-Pick C1-like 1 (NPC1L1), which is a molecular target of ezetimibe, a novel cholesterol lowering drug, plays a critical role in the cholesterol absorption. However, at the present time, there is little information about the molecular mechanism of NPC1L1-mediated cholesterol transport. Since, among nonsynonymous variants found in western people categorized as cholesterol low absorbers, six variants were located within 39 amino acids in the predicted extracellular loop of NPC1L1 protein, we hypothesized that this region may be important for the recognition of dietary cholesterol by NPC1L1. In the present study, as the first step to elucidate the cholesterol recognition by NPC1L1, we characterized the effect of these six variants on the NPC1L1 function by in vitro analyses. Expression vectors for the variants or wild-type of human NPC1L1 were constructed and transiently transfected to Caco-2 cells and hepatoma-derived McA-RH7777 cells, which revealed that four of the variants showed the reduced expression level and aberrant subcellular localization of NPC1L1 protein in both cell lines. Concerning the other two variants, which could not be distinguished from the wild-type NPC1L1 by the Western blot analysis and immunostaining, we constructed Caco-2 cells stably overexpressing the NPC1L1 variants and performed micellar cholesterol uptake assays. Although increases in the transport activities depending on the expression levels of introduced NPC1L1 and inhibitory effects of ezetimibe were observed also in the two variants, the relative activities were significantly lower compared with the wild-type NPC1L1. These results suggest that the reduction of intestinal cholesterol absorption in carriers of these six variants can be accounted for by the altered cellular localization or by the lower intrinsic activity of NPC1L1. In order to reveal the way NPC1L1 recognizes its substrates, further analyses using the two NPC1L1 variants with reduced intrinsic function may be useful.

01C09-4

INVESTIGATION OF THE IMPORTANCE OF TRANSPORTER-MEDIATED DRUG-DRUG INTERACTION FROM THE LITERATURE CLINICAL INFORMATION
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The importance of drug transporters in the pharmacokinetics and subsequent pharmacological effects has been gradually recognized by several human clinical studies. Drug-drug interaction mediated by transporters is one of the important situations causing the change in the transport function of transporters. Now we know that so many drugs are substrates for several drug transporters expressed in several tissues. Recently, several drugs, which are considered to be eliminated from blood circulation by extensive metabolism, are also substrates for hepatic uptake transporters (e.g. atorvastatin, cerivastatin, bosentan, repaglinide). In that case, if the uptake process is the rate-limiting step of the overall clearance of these drugs, the inhibition of hepatic uptake shows a greater impact on the pharmacokinetics of substrate drugs rather than the inhibition of metabolism. Therefore, to show the cases in which we must pay attention to the transporter-mediated drug-drug interaction by predicting these interactions quantitatively based on the pharmacokinetic theory, in the PK/PD seminar organized by Prof. Sugiyama, its members carefully searched the data of clinical drug-drug interaction studies, in which the pharmacokinetics of transporter substrate drugs was modulated by other drugs, and calculated the pharmacokinetic parameters of substrates and inhibitors. We also checked several parameters from in vitro experiments such as inhibition constant of inhibitors for each transporter and metabolic enzyme and predicted the maximum local protein unbound concentration of inhibitors at the target site. Based on these data, we supposed the major interaction sites and estimated the extent of the change in the plasma AUC of "victim" substrate drugs for each case. In our presentation, we will show you some key points for the prediction of clinical transporter-mediated drug-drug interaction by raising some examples analyzed.