CHARACTERISTICS OF SUBSTRATES UPTAKE INTO TRANSPORTER EXPRESSING OOCYTES
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[Introduction] Transporters are membrane proteins that play an important role for transportation of xenobiotics. Influx transporters have been evaluated using oocyte gene expression system, because of small background, high expression level, and large capacity. In the present study, we evaluated human organic anion transporters (OAT1, OAT3), human organic cation transporters (OCT1, OCT2, OCT3), human and rat organic anion transporting polypeptide (OATP1A2, OATP1B1, OATP1B3, Oatp1b2), and Na⁺-taurocholate cotransport protein (NTCP) using oocyte gene expression systems. Moreover, we examined the effect of OATP1B1 SNPs on the uptake of statins and sartans. [Method] Transporter genes were cloned from commercial cDNA library by PCR using gene-specific primers. cRNAs were synthesized by T7 polymerase and then injected into Xenopus laevis oocytes. After oocytes were incubated at 18°C for 3 to 7 days, uptake assay was conducted at room temperature. Uptake of radiolabeled substrates was determined by using a liquid scintillation counter, while non-radiolabeled substrates were determined by LC-MS/MS. [Results and Conclusion] All transporters were functionally expressed with high activity in Xenopus laevis oocytes. Calculated \(K_m\) values were comparable with the previous publications. Transport of statins and sartans was considerably diminished in OATP1B1*15 compared with that of wild type OATP1B1*1a. The oocyte gene expression system is useful for evaluating characteristics of drug uptake by transporters seen in various different organs such as the liver, kidney, and intestine. The system is also of use for studying altered pharmacokinetics as influenced by SNPs.

IMMUNOHISTOCHEMICAL LOCALIZATION OF AN ALDO-KETO REDUCTASE IN RAT TISSUES
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Enzymes belonging to the AKR1C subfamily in the aldo-keto reductase (AKR) superfamily exhibit oxidoreductase activities for endogenous steroids, and xenobiotic alcohols and carbonyl compounds. We previously characterized seven new members of the AKR1C subfamily that were found on rat genomic analysis. One of the enzymes, RAKc, shows much broader substrate specificity and high affinity for xenobiotic aliphatic, alicyclic and aromatic carbonyl compounds, compared to the other enzymes. Particularly, it efficiently catalyzes the reduction of toxic methylglyoxal, 2,5-hexanedione, and aldehydes (such as 4-hydroxynonenal) derived from lipid peroxidation. This enzyme is also unique in the abundant expression in rat lung. Here, we studied the localization of the enzyme in rat tissues by immunohistochemistry using specific antibodies against the enzyme, in order to obtain more insight of its function. In the lung, the immunostaining was detectable in the bronchiolar Clara cells and type II alveolar pneumocytes, but not in the ciliated cells. The immunostaining was also observed in the epithelial cells of the stomach and colon, and the brown adipocytes of the adipose tissues. Although no immunostaining was detectable in the parenchymal cells of the liver, proximal tubules of the kidney and the cells of the other tissues (brain, heart, esophagus, pancreas, spleen, muscle and skin), consistently pronounced staining was observed in the endothelium of blood vessels, where the enzyme and its mRNA were also detected. The results indicate a housekeeping function of RAKc in elimination of reactive products of lipid peroxidation and sugar metabolism, as well as its xenobiotic metabolism.