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Necessity of combined analyses tools for the understanding of chemical-induced toxicity; lithocholate as an example

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Considerable amounts of energy had been introduced to predict chemical-induced toxicity using so-called "omics" technologies. These new tools showed their power in data compilation, but the ample mounts rather hampered us to judge the outcome as toxic influences. In addition to demand of the well defined database of chemical toxicity, we are now seeking to refine technology for omics with the supports from related researches including drug/endobiotic metabolism and toxicokinetics.

Lithocholic acid is a naturally occurring hydrophobic bile acid and becomes high in the plasma ad liver during cholestasis. Unlike other bile acids, this bile acid is biotransformed in the body by enzymatic oxidation and conjugation in similar to xenobiotics and drugs. Lithocholate also behaves as receptor ligands. These data prompted us to investigate lithocholate-induced liver damage as an endogenous-model of drug-induced toxicity. Feeding of lithocholic acid in C57BL6 mice induced perturbation of lipid metabolism and in later resulted in the appearance of liver disorders: as follows. 1% Lithocholate-feeding had no clear influence on biochemical parameters in the first day. Serum bile acid-level started to increase at third day after the administration. Liver diagnostic parameters became high at the fifth day and necrotic change appeared at the 7th day. Chemicals taken in the body elicit both adaptive response and initiation of deteriorating events in parallel. We first looked at the capabilities of protection in ways of analyses of disposition and metabolism. Clear increases of tauro conjugates of chenodeoxycholate (7alpha-oxidized) and lithocholate were detected in the liver. These metabolites are excreted in the bile, but not in urine. Hepatic level of total lithocholate rather than chenodeoxycholate or deoxycholate correlated with serum ALT and AST.

In our previous studies, FXR(farnesoid x-receptor)-null mice with reduced Bsep (Bile salt excretory pump) capacity rather showed resistance to lithocholate-induced liver damages. This phenomenon is explained by the clear enhanced abilities of St2a-mediated sulfation of taurolithocholate in the null mice (J. Biol. Chem. 2003) and by the lesser efficacy of entero-hepatic circulation of the

sulfoconjugate than of the non-sulfoconjugate (xenobiotica 2006). This was further supported by the reversal of protective phenotype in Fxr-null mice after the administration of dehydroepiandrosterone as a competitive inhibior of lithocholate sulfation (DMPK 2007). In wild-type C57BL6 mice fed lithocholate, hepatic capacities of sulfation (St2a) as well as oxidation (Cyp3a) are maintained. Co-administration of PXR (pregnane x-receptor) ligand, pregnenolone 16alpha-carbonitrile (PCN) from the fourth day abolished clearly the raise of toxic signs. These changes are consistent with the induction of St2a, suggesting the involvement of enhanced sulfation of lithocholate in the PCN-mediated protection. Observed drastic effects, however, suggest the additional mechanism for the protection of PCN-treatment.

Reduced flow of bile is one of typical properties in lithocholate-fed animals. Although no changes are observed on serum and hepatic cholesterol levels, clear drop in hepatic, but not serum, levels of triglyceride and fatty acid were detected. These results suggest the disturbances of phospholipids metabolism in lithocolated-treated mice. Thus, we investigated the time-dependent profile of gene expression using a microarray technique.

Profiles of various functional genes such as nuclear receptor-mediated signaling, Cyp and other drug metabolizing enzymes, Abc and Slc transporters are compiled. These data suggest a clear switching of fatty acid biosynthesis and metabolism, suggesting the competition between energy production and phospholipids excretion in the liver. Reversal of these papameters to the normal direction after PCN-treatment imply the stimulation of TG-phospholipid production as a protective role of PCN.