【P-16】

Hepato-toxicological Evaluation of Alpha-naphthylisothiocyanate(ANIT) Using Toxicogenomics

Seokjoo Yoon, Ji-Yoon Hwang, Jung-Sun Lim, Sun-Young Jung, Han-Jin Park, Jae-Woo Cho, Yong-Bum Kim, Moon-Koo Chung and Sang-Seop Han
Korea Institute of Toxicology, KRICT, Daejeon, Korea

In this study, we used α-naphthylisothiocyanate (ANIT) as hepatotoxicant. We can found typical hepatocyte and bile duct damage with ANIT treatment. Many scientists devoted to elucidated mechanism of ANIT effect on liver for many decades. In toxicogenomics research, ANIT is invaluable hepatotoxicant. Since its abundunt toxicological data could support toxicogenomics study. We adminstered ANIT to 12-week-old male C57BL/6 mice with control(corn oil only), low dose (6 mg/kg), mid dose (30 mg/kg) and high dose (60 mg/kg) via gavage.. After 2, 4, 8 and 24 hrs treatment, mice were sacrificed. Livers were surgically removed (for RNA and histopathological study (H&E, PAS, Reticulin). Also blood was collected for blood biochemical analysis (ALT, AST, ALP). Liver specimens were fixed in 10% neutrally buffered formalin and paraffin embedded. Deparaffinized sections were stained and analyzed bu light microscopy. Serum ALT, AST, ALP were analyzed with commercial automatic blood chemical analyzer. Total RNA isolated following manufacturer's instruction. Quality was checked via Agilent 2100 bioanalyzer and quantity was determined based on 260nm absorbance. Qualified RNA samples were proceeded for next step. Quality control also conducted at cDNA and cRNA synthesis step. Affymetrix oligonucletide chips were used for microarray experiments. Test arrays were used for hybridization validation before main chip experiments. After DNA chip experiments, all gene data were analyzed with stastical tool and clusterd with SOM, hierachichal clustering and visualized using the TreeView program. As a result of blood biochemical analysis, there were no significant changes in 2, 4, 8 hr after ANIT treatment. High increase of the serum concentration of AST and ALT were observed in 24h after high dose (60 mg/kg) of ANIT treatment. Most of doses and time points were not showed significant changes in histopathological examination. But, some hepatocellular necrotic foci were observed and inflammatory cell infiltration into bile duct and necrotic area in 24h after mid dose (30 mg/kg) of ANIT treament. In 24hr after high

dose of ANIT treatment, multiple necrotic foci were found with PAS staining examination. In reticulin stain examination, reticulin fiber was collapsed by hepatocellular necrosis in the group. Cluster analysis for gene expression patterns were conducted by SOM (Self Organizing Map) and Hierachical clustering. These data will give great potential to new drug and new toxicological biomarker development.

Keyword: DNA chip, ANIT, Cholestasis, Histopathology