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Prediction of drug-induced hepatotoxicity by LC-MS analysis of urinary metabolites.

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Multivariate pattern recognition analysis combined with liquid chromatography-mass spectrometry was employed to test the feasibility to predict chemical-induced hepatotoxicity in rats. Urine samples from hepatotoxin treated groups and control vehicle treated group were analyzed by gradient HPLC coupled with electrospray mass spectrometry. The principal component analysis (PCA) of urine samples enables the samples to be clustered according to each hepatotoxin or control groups in PC maps. To predict drug-induced hepatotoxicity, statistical models were constructed using linear discriminant analysis and soft independent modeling of class analogy with residual distances to the model (SIMCA-RD). Four different classes of hepatotoxin could be distinguished with 96.7% predictability by SIMCA-RD method.

Results and Discussion

Toxicants disrupt a complex network of metabolic interrelationships, leading to significant changes in concentrations of a large number of constituents in body fluids, which is not easily attainable by manual data treatment. Therefore systematic approaches are required for studying in vivo metabolic profiles. Metabonomics, where metabolite profiling is combined with chemometric analysis, thus is a rapidly growing area of scientific research. Metabonomic approach is now being investigated by large pharmaceutical companies to screen compounds for toxicity, lead compound selection. In metabonomics the effect of a pharmaceutical candidate on a whole animal or organ is investigated by measuring the changes in endogenous metabolites over a time course following administration of a compound.

To investigate the complex metabolic consequences of toxic reactions in biological systems, information-rich analytical approaches are required. Liquid chromatography-Mass

spectrometry (LC-MS) has become the technique for bioanalysis, both quantitative and qualitative. Simple unsupervised principal component analysis (PCA) of the analytical data enables the visualization of biological datasets based on the inherent similarity/dissimilarity of samples with respect to their biochemical composition. Once a relationship between metabolic profiles and toxicity has been identified from the initial analysis, supervised approaches such as linear discriminant analysis (LDA) or soft independent of class analogy with residual distance to the model (SIMCA-RD) perform to maximize the separation between the classes and to construct representative models of toxicity. In addition, data manipulation might be preprocessed to optimization metabonomic analysis. In this study, pattern recognition analysis combined with LC-MS was employed to test the feasibility to predict chemical-induced toxicity in rats using four model hepatotoxins: α-naphthylisothiocyanate (ANIT), carbontetrachloride, acetaminophen and diclofenac.

Urine samples from hepatotoxin treated groups and control vehicle treated group were analyzed by gradient HPLC coupled with electrospray mass spectrometry. LC-MS based profiles of urine samples showed different levels of endogenous metabolite that were characteristic for each hepatotoxin. In PC maps generated by PCA to evaluate time-dependent metabolic variations in rats treated with ANIT, the metabolic trajectory of the ANIT treated samples coordinates moved away from the predose position, reached a maximum separation 32-48 hr. The metabolic profiles were partially recovered to the basal control conditions in day 7 after dosing. PCA were performed on dataset from four hepatotoxin treated groups and control group collected at 32-48 hr to investigate toxin-related metabolic variations. Samples formed a distinct and isolated cluster according to each hepatotoxin and control group in the PC maps, indicating drug-induced perturbation in the urine profiles. To construct models for hepatotoxin, supervised analysis such as LDA and SIMCA-RD were performed. Among the methods, SIMCA-RD showed the highest predictability over 95% of the results of cross validation using leaving-one-out method. These results suggest that LC-MS-based metabonomic approaches might be a useful tool for the prediction of drug-induced hepatotoxicity.