[sPHT-5] [11/15/2007 (Thr) 14:40 - 15:20 / 2nd FL]

Application of metabonomics in development of pharmaceuticals

Takashi Yamoto, Yosuke Ando, Naoki Kiyosawa, Sunao Manabe

Medicinal Safety Research Laboratories, Daiichi Sankyo Co., Ltd.

Keywords: hepatotoxicity, acetaminophen, hepatic glutathione depletion model, L-buthionine (*S*, *R*)-sulfoximine

Introduction

It is important for drug development to have a balance between drug efficacy, pharmacodynamics (PD), and safety, whereas the emphasis in drug screening has been placed on the pharmacological efficacy in the early stages. However, the importance of early stage screening of PD and safety is recently recognized from the accumulated experience of drug development halted in late stages by unacceptable PD and safety profiles. In the PD screening, prediction of CYP induction/inhibition or drug absorption has been improved with establishment of methodology. Thus as the next step, improvement of toxicity evaluation/prediction in the early stages of drug development is crucial to achieve better safety assessment in humans as well as reduction of time and costs for the drug development in pharmaceutical companies. The omics technology, genomics/ proteomics/ metabonomics (toxicotrinomics), is a promising tool for efficient and reliable toxicity screening.

Metabonomics is an emerging tool in the omics technologies to detect the metabolites' dynamics in the biological fluids such as urine or blood samples. By

integrating the metabonomics information with genomics and proteomics data, it is expected that we can identify and utilize novel biomarker(s) for assessing and predicting toxicities. Since metabonomics study requires less- or non-invasive sampling from the body, it is expected that application of the biomarker(s) identified from experimental animals to clinical trials will be much easier than with other techniques. However, metabonomics analysis requires expensive facilities to achieve sufficient sensitivity and specificity, as well as highly specialized analytical knowledge in addition to biological skills.

Our Experience: Exploring urine biomarker candidates for hepatotoxicity and hepatic glutathione depletion using UPLC-TOF/MS

In the symposium, we will present two examples of studies where candidate biomarker sets for assessing hepatotoxicity and hepatic glutathione depletion were identified using metabonomics technique.

Study design and metabonomics data processing

Male F344/DuCrlCrlj rats were used in both the acetaminophen (APAP)-induced hepatotoxicity model study and the L-buthionine (*S*, *R*)-sulfoximine (BSO)-induced hepatic glutathione depletion model study. Animals were placed into individual metabolism cages for 24 hours to collect the urine samples. Food and water were available *ad libitum*. The urine samples were collected into tubes containing 1 ml of 1% sodium azide and maintained below 0°C (on dry ice). The urine samples were centrifuged to remove debris, diluted 4-fold by deionized water, and injected onto an ultra performance liquid chromatography-time-of-flight/mass spectrometer (UPLC-TOF/MS, Waters Corporation) for metabolite profiling. The data were collected in positive ionization mode. The UPLC-TOF/MS data were analyzed

using the software Micromass MarkerLynx Applications Manager Version 1.0 (Waters Corporation). The processed data were analyzed by principal component analysis (PCA).

APAP-induced hepatotoxicity model

Male 9-week-old F344/DuCrlCrlj rats (n=4/group) were treated orally with single doses of 0, 100, 500, or 800 mg/kg of APAP and urine samples were collected 24 hours after the treatment.

By the UPLC-TOF/MS data analysis, a total of 1,372 peaks were detected in the control group. We selected 175 metabolite peaks that were correlated with plasma alanine aminotransferase (ALT) level by Spearman's rank correlation test ($p<0.05$) and plotted each sample (Scores plot) and each peak (Loadings plot) by PCA. The Scores plot shows that each dosage group was clearly clustered which also reflected the increased levels of plasma ALT (Fig. 1). The identified metabolite peaks are expected to be associated with the progression of APAP hepatotoxicity. By the Loadings plot, metabolite peaks that strongly correlated with plasma ALT elevation could be selected (Fig. 2). These metabolite peaks are expected to be biomarker candidates for assessing the APAP-type hepatotoxicity of chemicals.

·*PCA (Scores plot):* Plot of each sample

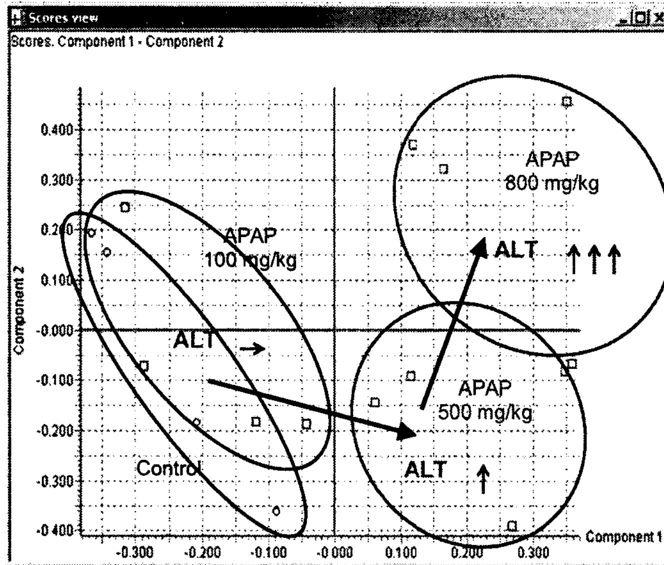


Fig.1: Scores plot of APAP-induced hepatotoxicity model
Cluster of each group was clearly correlated with plasma ALT levels

·*PCA (Loadings plot):* Plot of each peak

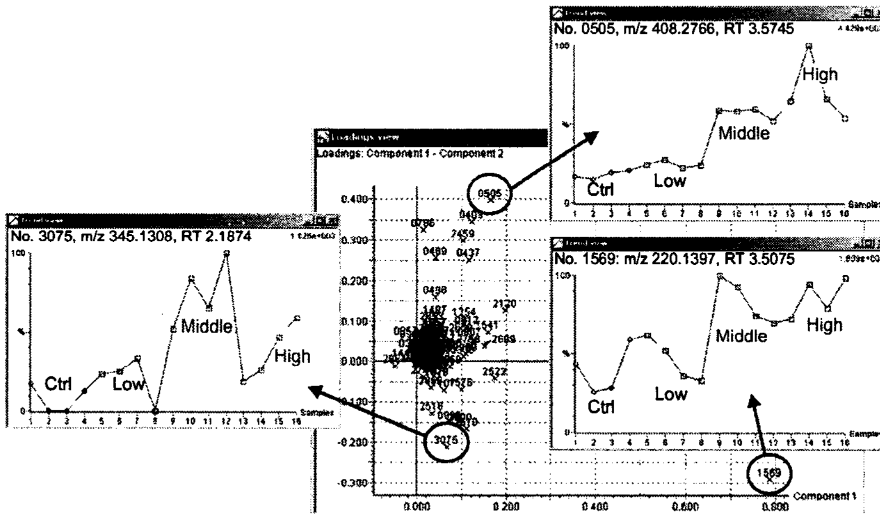


Fig.2: Loadings plot of APAP-induced hepatotoxicity model
Endogenous metabolite peaks that contributed strongly to the clustering could be selected.

BSO-induced hepatic glutathione depletion model

Male 9-week-old F344/DuCrlCrlj rats (n=3/group) were treated with 0 or 20 mM of BSO in drinking water for 4 days and urine samples were collected continuously for 24 hours at the end of BSO-treatment.

By the UPLC-TOF/MS data analysis, we selected 105 peaks that showed inverse correlation ($r<-0.8$ by Pearson's correlation) to hepatic glutathione (GSH) level from the total of 1,372 peaks identified in the APAP-induced hepatotoxicity model study. Both BSO-treated groups and APAP (500 and 800 mg/kg)-treated groups were each classified into the common cluster in the Scores plot (Fig. 3). Metabolite peaks that contributed to clustering are suggested to be endogenous metabolites responding to GSH depletion in the liver of APAP-treated rats. By the Loadings plot of PCA, endogenous metabolite peaks that correlated with GSH level were identified (Fig. 4). These metabolite peaks are expected to be biomarker candidates for assessing the GSH depletion in the liver of APAP-treated rats.

·PCA (Scores plot): Plot of each sample

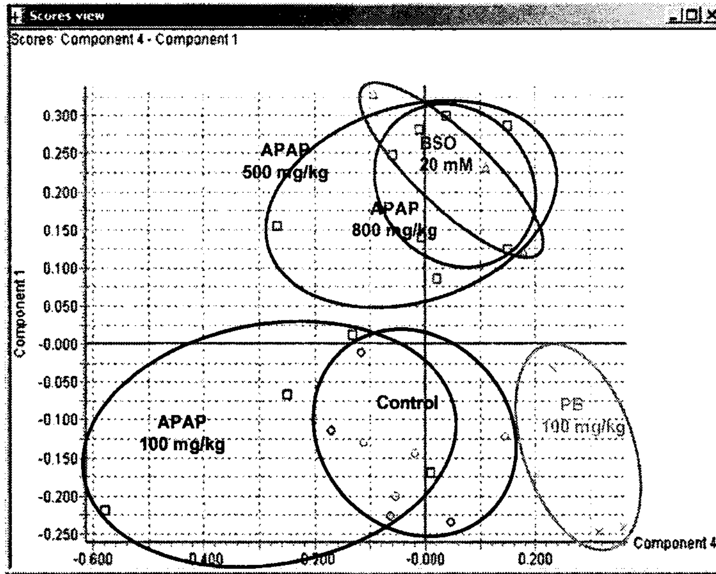


Fig.3: Scores plot of BSO-induced hepatic glutathione depletion model
BSO- and APAP-treated groups formed a common cluster.
PB: phenobarbital

·PCA (Loadings plot): Plot of each peak

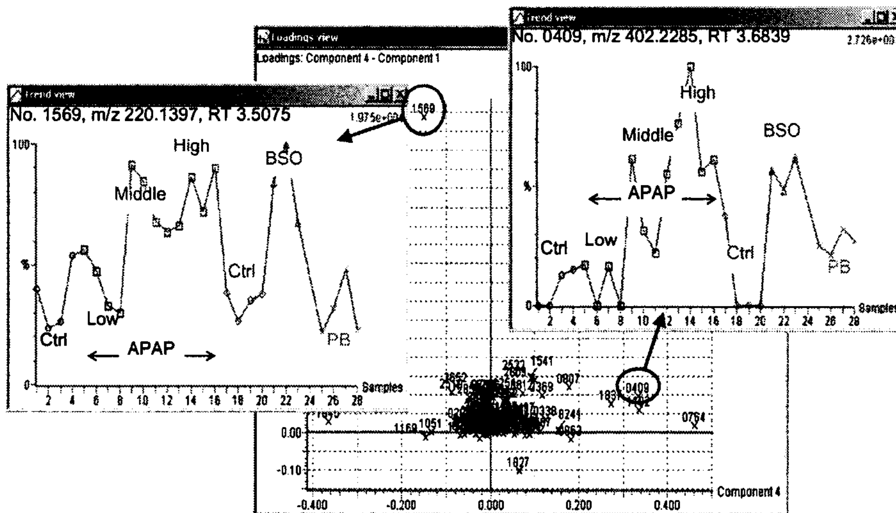
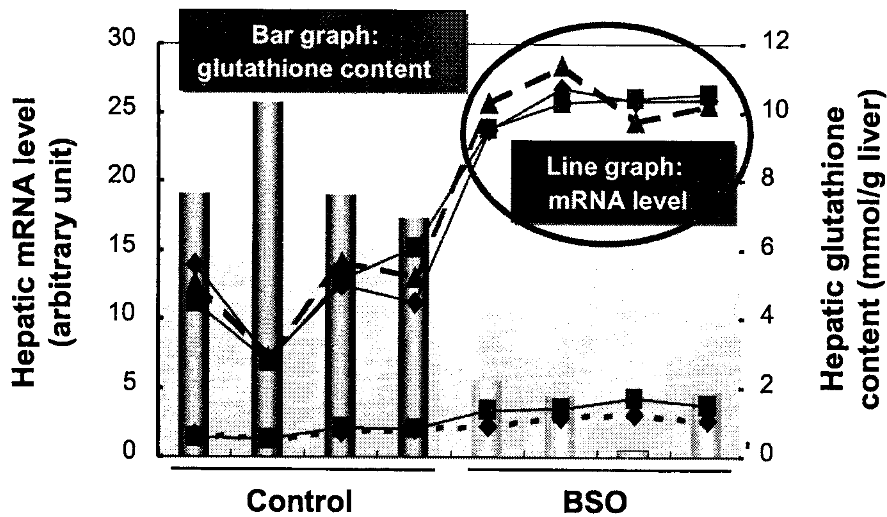


Fig.4: Loadings plot of BSO-induced hepatic glutathione depletion model
Endogenous metabolite peaks correlated with GSH reduction and contributed to the clustering strongly could be extracted.

Comparison between toxicogenomics and metabonomics data in BSO-induced hepatic glutathione depletion model

Metabonomics data in the BSO model were compared with our previous toxicogenomics data (Kiyosawa et al., 2004). As shown in Fig. 5, 69 gene probe sets of the Affymetrix GeneChip™ that showed inverse correlation to hepatic GSH level were selected. A PCA using these GSH depletion related gene probes successfully illustrated the time-course of hepatic gene expression after the treatment with APAP, phenobarbital (PB) or clofibrate (CPIB), and the expression profiles were thought to reflect the changes in hepatic glutathione levels (Fig. 6). Using appropriate selection criteria and algorithms, sensitive and specific biomarkers can be extracted for metabonomics in the same way as for genomics biomarkers. Metabonomics biomarkers have an advantage, considering the requirement for less- or non-invasive sampling from the body.

We are continuing to explore metabonomics biomarker(s) using the well studied toxic compounds to construct an in-house database and we will apply these methods to safety assessment in the near future.



Selection Criteria
Spearman's correlation (P<0.05)
Pearson's correlation coefficient (r<-0.80)

69 genes were identified

Fig.5: Identification of GSH depletion-responsive genes

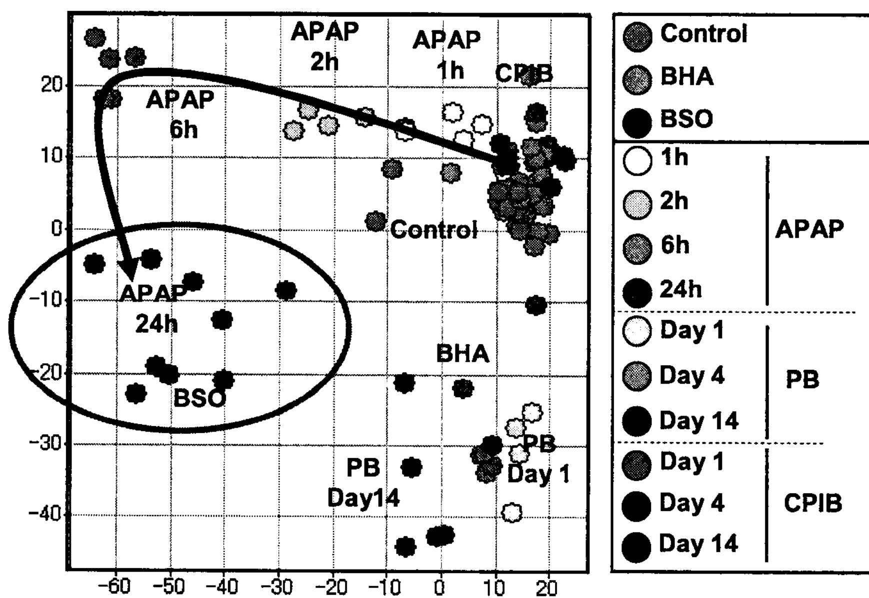


Fig.6: PCA using GSH depletion-correlated gene expression markers

BHA: butylated hydroxyanisole

Conclusion

To utilize the toxicotrinomics data for toxicity screening in the early stages of drug development and in toxicity studies, it is imperative to construct a large-scale reference database (DB) and to standardize the data acquisition and analytical procedures. In addition, overcoming data variability derived from inconsistent platform and facility differences is a crucial challenge. To apply the omics techniques for toxicity screening in the early stages and toxicity studies, we must compensate for the mutual drawbacks they contain by taking the best advantages of each omics technique. In Europe, the InnoMed/PrdTox consortium is now selecting 14 compounds that were abandoned at different development stages due to hepato- or nephrotoxicity. The project involves the construction of a relational DB combining clinical pathology and toxicological pathology with information from new technology, i.e. toxicogenomics, proteomics, and metabonomics. In the USA, the liver toxicity biomarker study (LTBS) consortium is in the ongoing process of selection 5 pairs of toxic/ non-toxic compounds and will construct an integrative DB. Integration of the omics techniques with traditional toxicity evaluation will lead to better safety assessment of drugs in drug development.