REVIEW

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Metabonomics for discovering biomarkers of hepatotoxicity and nephrotoxicity

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Metabonomics has played increasingly important roles in pharmaceutical research and development. Safety assessment of drugs is a key stage in drug development and one which represents a significant attritional hurdle. However, characterization of the molecular mechanisms of drug toxicity still remains an enormous challenge. Recent advancements in 'omics' sciences, and in particular metabonomics, has enabled some elucidation or insights into toxicological sequelae. Metabonomics is a global metabolic profiling framework which utilizes high resolution analytics together with chemometric statistical tools to derive an integrated picture of both endogenous and xenobiotic metabolism. Hepatotoxicity and nephrotoxicity are major reasons that drugs are withdrawn post-market, and hence it is of major concern to both the Food and Drug Administration and pharmaceutical companies. There is a strong need to develop reliable biomarkers that can accurately predict toxicity in the drug discovery and development process and are translatable to the clinic. A deeper understanding of global perturbations in biochemical pathways and useful biomarkers could provide valuable insights about mechanisms of toxicity. This review summarizes some current progress in the application of metabonomic in understanding drug-induced hepatotoxicity and nephrotoxicity, with an emphasis on identifying early toxicity biomarkers.

1. Introduction

The primary definition of metabonomics, as "the quantitative measurement of the dynamic multiparametric metabolic response of living systems to pathophysiological stimuli or genetic modification" (Nicholson et al. 1999), has recently demonstrated significant potential in many fields such as responses to environmental stress (Workentine 2010), comparing mutants (Johansen et al. 2009), drug metabolism (Li et al. 2010), drug discovery (Wei 2011), toxicology (Aoki et al. 2011; Want et al. 2010), nutrition (Pexa et al. 2008; Astle et al. 2007), genetic manipulation (Urakami et al. 2010), cancer (Urayama et al. 2010; Sreekumar et al. 2009), comparing different growth stages (Wang et al. 2009a), diabetes (Lanza et al. 2010; Bao et al. 2009), natural product discovery (Lawaetz et al. 2009), traditional medicine (Zhang et al. 2010; Wang et al. 2011a) and epidemiology (Bictash et al. 2010). Unique biochemical molecules generated in a living system involve measuring low molecular-weight metabolites in complex biofluids/tissues to study perturbations in response to physiological challenges, toxic insults or disease processes (Watkins and German 2002). Metabonomics has the potential to enable mapping of early biochemical changes in toxicity and hence provide an opportunity to develop predictive biomarkers that can trigger earlier interventions (Ebbels et al. 2007; Bodor and Buchwald et al. 2010). Recently, metabonomics have emerged as a new and promising 'omics' platform that shows potential in biomarker discovery, especially in areas such as disease diagnosis, assessment of drug efficacy or toxicity.

Hepatotoxicity and nephrotoxicity are two major reasons that drugs are withdrawn post-market, and hence it is of major concern to both the FDA and pharmaceutical companies (Wang et al. 2011b). Lack of efficient and accurate biomarkers has become a leading cause of severe injury disease and therefore poses a major clinical challenge. Therefore, there is need for new efficient biomarkers that are demanded to reduce the time and cost of drug discovery and development. Metabonomics could play a key role in providing novel and specific biomarkers for injury and for predicting toxicity (Russmann et al. 2009). The number of cases of adverse drug reactions (ADRs) in marketed drugs has climbed faster than the number of total drug prescriptions issued. Minimizing the financial cost is one of the most important aims of a pharmaceutical discovery programme. However, diagnostic and monitoring tools for hepatotoxicity and nephrotoxicity are currently inadequate. Current emphasis on efficient screening of novel therapeutic agents in toxicological studies has resulted in the evaluation of novel analytical technologies (Holmes et al. 2001). Novel technologies that increase the probability of making the right choice early save resources, and promote safety, efficacy and profitability. Fortunately, metabonomics is a systems approach for studying *in vivo* metabolic profiles, which promises to provide information on drug toxicity in the discovery-and-development process (Nicholson et al. 2002). It was able to more sensitively detect changes related to

toxicity and discover novel markers. Over the past ten years, the international literature demonstrates a growing interest for this technology and its capabilities (Boudonck et al. 2009). Detection of the systemic toxic effects could be obtained with metabonomics at an earlier stage compared to the clinical chemistry and histopathological assessment.

Metabonomics will provide a better understanding of the mechanism of toxicity and may facilitate the prediction of toxicity of unknown compounds (Stierum et al. 2005). Mechanism-based markers of toxicity can be discovered and marker metabolites can be therapeutic targets as well (Arakaki et al. 2009). Biomarkers are widely used in clinical practice for the diagnosis, assessment of severity and response to therapy in a number of clinical disease states (Mamas et al. 2011; Heinzmann et al. 2010). Metabonomics driven top-down systems biology can be used for identifying biomarkers of toxicity and be able to provide comprehensive information on the dynamic process of drug toxicity. Precise identification and accurate quantification of metabolites facilitate downstream pathway and network analysis using software tools for the discovery of clinically accessible and minimally invasive biomarkers of drug efficacy and toxicity. However, optimal biomarker information still pose significant challenges in complex mixture analysis for improved biomarker identification (Fonville et al. 2010; Niemann et al. 2007). A future hope for the metabonomic approach is the identification of biomarkers that are able to highlight individuals likely to suffer from drug-induced toxicity and enable early diagnosis or the identification of those at risk. With the expected surge in the scope and quality of metabonomic measurements, metabonomics is destined to play an even more central role in the near future as an efficient diagnostic tool and as a safety evaluator of drug candidates. In this review, we present and discuss the current progress in the application of metabonomics to drug safety and with particular emphasis on recent success in the discovery of toxicity biomarkers for hepatotoxicity and nephrotoxicity.

2. Potential role of metabonomic biomarkers

Metabonomics has been fueled by the development of experimental platforms such as GC/MS, LC/MS and NMR that are capable of accurately measuring hundreds of small molecule metabolites in biological samples (Athersuch et al. 2010; Barton et al. 2010; Johnson et al. 2010). It allows the simultaneous and relative quantification of thousands of different metabolites within a given sample using sensitive and specific methodologies, typically in the discovery of new diagnostic biomarkers for toxicity. Metabolites identified will need to form the basis of larger, prospective, externally validated studies in clinical cohorts for their future use as biomarkers. Subsequent statistical modelling of the complex multivariate spectral profiles enables discrimination between phenotypes of interest and identifies panels of discriminatory metabolites that represent candidate biomarkers. Useful biomarkers of toxicity should be present in biofluids such as blood or urine to allow minimally invasive collection and rapid quantitation, should sensitively and reproducibly indicate potential adverse health effects prior to irreversible tissue damage, toxicity, or disease onset. Currently, a number of novel biomarkers have been made the transition to routine use in clinical practice, widely used for the diagnosis, assessment of toxicity and response to therapy (Denery et al. 2010).

Metabonomics driven top-down systems biology can be used for identifying biomarkers of toxicity. However, optimal biomarker information still pose significant challenges in complex mixture analysis for improved biomarker identification (Fonville et al. 2010). Specific and sensitive biomarkers constitute the missing link in the continuum of exposure to toxins and susceptibility disease development as well as possible therapeutic intervention (Niemann et al. 2007). Important requirements for biomarker development are a detailed understanding of biochemical pathways involved in hepatotoxicity, nephrotoxicity, minimal invasiveness and capacity to screen large at-risk populations. Biomarkers should be organ specific and equally applicable in clinical care of patients. Metabonomics has been applied to define biomarkers related to prognosis or diagnosis of a disease or drug toxicity/efficacy and in doing so hopes to provide greater pathophysiological understanding of disease or therapeutic toxicity/efficacy (Lin et al. 2011). Deeper understanding of global perturbations in biochemical pathways could provide both valuable insights about underlying mechanisms and new information on pathways and processes of toxicity. Future hope for the metabonomic approach is the identification of biomarkers that are able to highlight individuals likely to suffer from drug-induced toxicity and enable early diagnosis or the identification of those at risk.

3. Metabonomic evaluation of hepatotoxicity

The liver is the primary organ responsible for phase I and phase II drug metabolism and for processing both endogenous and xenobiotic substance. Evaluation of potential hepatotoxicity represents a critical step in the development of new drugs. Hepatotoxicity may be the result of the drug itself or, more frequently, a result of the bioactivation process and the production of reactive metabolites. Metabonomics facilitates exploring hepatotoxicity and clinical investigations of liver disease (Masson et al. 2010). Metabonomics are increasingly used by the pharmaceutical industry for the screening of the hepatotoxic potential of new molecules. Biomarkers have notably contributed to the understanding of mechanisms responsible for hepatotoxicity. Assessment of current hepatotoxicity is limited by the inability to measure a wide spectrum of potential mechanistic changes involved in the drug-induced toxic injury. Fortunately, metabonomic approaches can increase the sensitivity of detection and provided new insight in the mechanisms of hepatotoxicity and help to define patterns of hepatotoxicity for early identification of potential adverse effects of the drug to the liver. Therefore, metabonomic approaches have been used in preclinical studies of compounds that cause hepatotoxicity, and accelerate the drug development process.

A nontargeted metabolic profiling method has been used for evaluating the hepatotoxicity of valproic acid (Lee et al. 2010). It was demonstrated that the metabonomic approach had great potential for predicting valproic acid-induced hepatotoxicity and discovering novel biomarkers, 8-hydroxy-2'-deoxyguanosine and octanoylcarnitine. Metabonomics could provide a powerful and efficient technical platform to characterize acetaminophen (APAP)-induced toxicity through identifying novel biomarkers and unraveling novel mechanisms (Chen et al. 2009). Subsequent metabolic consequences contributing to APAP-induced hepatic necrosis and apoptosis had been fully elucidated. A dose-response study showed that the acylcarnitines in serum contribute to the separation of wild-type mice undergoing APAP-induced hepatotoxicity from other mouse groups in a multivariate model. Distinct from serum aminotransferase activity and hepatic glutathione levels, the pattern of serum acylcarnitine accumulation suggested that acylcarnitines can function as biomarkers for monitoring APAP-induced hepatotoxicity. It revealed that metabolic activation and oxidative stress following APAP treatment can cause irreversible inhibition of fatty acid oxidation, potentially through suppression of PPAR alpha-regulated pathways. Metabonomic profile of

urine from rats administrated with rifampin revealed that hepatic toxicity induced by rifampin is related to the reduction of energy metabolism in tricarboxylic acid cycle and the perturbation of glucose and lipid metabolism (Liao et al. 2008). This was consistent with the results of traditional toxicity evaluation measurements. Metabolites in plasma were analyzed in relation to changes in rats that received bromobenzene to induce acute hepatic centrilobular necrosis (Heijne et al. 2005). Multivariate statistical analysis showed that metabolite profiles of blood plasma were largely different from controls when the rats were treated with bromobenzene. Levels of endogenous metabolites like alanine, lactate, tyrosine and dimethylglycine differed in plasma from treated and control rats, providing putative novel markers of hepatotoxicity and including the involvement of apoptosis and changes in glycolysis and amino acid metabolism. Waters et al. (2001) used metabonomics to detect novel biomarker and metabolite information, implicating specific putative protein targets in the toxicological mechanism of bromobenzene-induced centrilobular hepatic necrosis. In addition to a holistic view of the effect of hepatic toxicity on the metabolome, a number of putative protein targets of bromobenzene and its metabolites were identified including those enzymes of the glutathione cycle, exemplified by the presence of a novel biomarker, 5-oxoproline, in liver tissue, blood plasma, and urine. As such, metabonomics technology in resolving the mechanistic complexity of drug toxicity as well as the benefits of frontloading this approach in drug safety evaluation and biomarker discovery. Recently, a metabonomic approach using NMR spectroscopy was adopted via pattern recognition by using Principal component analysis (PCA) to explore the possible hepatotoxic mechanisms of combined exposure to polychlorinated biphenyls and 2,3,7,8-tetrachlorodibenzo-p-dioxin (Lu et al. 2010). The loadings plots of the PCA revealed remarkable increases in the levels of lactate, glucose, taurine, creatine, and 2-hydroxyisovaleric acid and reductions in the levels of 2-oxoglutarate, citrate, succinate, hippurate, and trimethylamine-N-oxide in rat urine after exposure. The changed metabolites may be considered possible biomarker for the hepatotoxicity. The findings shows that mitochondrial dysfunction and fatty acid metabolism perturbations might contribute to the hepatotoxicity. Liao et al. (2007) have investigated the effect of different treatment periods, of isoniazid on the metabonomic profile of rat urine and its relationship to traditional toxicity evaluation of blood biochemical indicators and histopathology and to explore the feasibility of metabonomics in the application of drug toxicity. It could been shown a trajectory bias from those of the controls or pre-administration, and such bias exaggerated along with the prolongation of treatment, indicating a more servere toxic injury, which helps track and identify biomarkers. The hepatic toxicity induced by isoniazid is related to the injury of mitochondrial function, reduction of energy metabolism in tricarboxylic acid cycle, and perturbations in the metabolism of glucose and lipid. Therefore, metabonomics can be recognized as an ideal technique to explore and evaluate the drug toxicities. Investigations into the hepatotoxicity of Bay41–4109 have been performed by metabonomics using high-resolution NMR (Shi et al. 2007). The predominant changes identified in liver tissue aqueous extracts included an increase in the signal intensities of lactate, 3-amino-isovalerate, pyruvate, choline, trimethylamine-N-oxide and a reduction in the intensities of taurine, hippurate and D-glucose. In liver tissue chloroform/methanol extracts, there was a remarkably increase in many of the lipid signals including the triglyceride terminal methyl, and methylene groups. These observations all provide evidence that fatty acid metabolism disorder and mitochondrial inability might contribute to the hepatotoxicity of Bay41–4109. The metabonomic effects of hepatotoxic doses of pravastatin on the urinary metabolic profiles of female rats have been examined using both NMR and UPLC/MS analysis highlighting trajectory in the urinary metabolite profiles (Lenz et al. 2007). The markers, which included elevated taurine and creatine, as well as bile acids, were consistent with hepatotoxicity in some animals, and this hypothesis was supported by histopathological and clinical chemistry findings. Craig et al. (2006) investigated the mechanism of toxicity of methapyrilene to rats causing periportal liver necrosis utilizing an metabonomics with NMR spectroscopy. The data further define the changes that occur in metabolic pathways during methapyrilene hepatotoxicity, revealing modification of biomarkers associated with oxidative stress and a change in energy usage. A novel small molecular compound, 3*Z*-3-[((1)*H*-pyrrol-2-yl)-methylidene]-1-(1-piperidinylmethyl)-1,3-2*H*-indol-2-one (Z24) can inhibit angiogenesis in new blood vessels. The hepatotoxicity effects and ajectory analysis of Z24 have been conducted in female Wistar rats by using metabonomic analysis of NMR spectra of urine, plasma and liver extracts (Wang et al. 2006). Moreover, the most notable effect of Z24 on the metabolism was the reduction in the urinary levels of creatinine and TMAO and the increase in acetate, citrate, succinate and 2-oxo-glutamate with time dependence. Results illustrated that in rats Z24 inhibits mitochondrial function through altering the energy and lipid metabolism, which results in the accumulation of free fatty acids and lactate because of the lack of aerobic respiration. The cause of idiosyncratic hepatotoxicity was reported to be that environmental factors such as concurrent inflammation initiated by bacterial lipopolysaccharide increase an individual's susceptibility to drug toxicity. Ranitidine, a histamine-2 receptor antagonist, causes idiosyncratic liver injury in humans. Maddox et al. (2006) conducted a study to distinguish animals cotreated with lipopolysaccharide and ranitidine from those treated with each agent individually. PCA of urine spectra by either NMR or mass spectroscopy produced a clear separation of the rats treated with lipopolysaccharide/ranitidine from the other groups. Clinical chemistry and histopathology corroborated these results. These findings support the potential use of a noninvasive metabonomic approach to identify drug candidates with potential to cause idiosyncratic liver toxicity with inflammagen coexposure. Ishihara et al. (2009). have concluded that bile acids, valine and methyl malonate have a possibility to be urinary cholestatic biomarkers, which distinguish a difference in mechanism of toxicity. So, metabonomics thus appears to be useful for determining the mechanisms of toxicity and can be front-loaded in drug safety evaluation and biomarker discovery. In a previous liver toxicity study, Lv et al. (2010) used global metabonomics to characterize phenotypically biochemical perturbations and the potential mechanisms of the gentamicin-induced liver toxicity. The mass spectra signals of the detected metabolites were systematically deconvoluted and analyzed by pattern recognition analyses, revealing a time-dependency of the biochemical perturbations induced by gentamicin toxicity. It showed that several metabolites involved in creatine, nicotinic acid, prostaglandin E2, and cholic acid were identified and validated as phenotypic biomarkers of gentamicin induced toxicity. Using LC/MS metabonomics demonstrating increased levels of conjugated bile acids in response to individual compounds, provide earlier detection of toxicity as compared to conventional parameters, and may allow distinction of different types of hepatobiliary toxicity (Ellinger-Ziegelbauer et al. 2011). Investigations into the safety biomarker for atorvastatin have been performed via metabonomic studies, supporting its application for liver toxicity treatment and prevention (Kumar et al. 2010). Estrone, cortisone, proline, cystine, 3-ureidopropionic acid and histidine confirmed by targeted metabolic profiling were proposed as potential safety biomarkers related with the liver toxicity of atorvastatin. A metabonomics-based systems

toxicology approach was used to profile the urinary metabolites for the toxicity related processes and pathogenesis induced by doxorubicin to rats (Wang et al. 2009b). Various endogenous metabolites involved in the toxic processes could be identified using their accurate mass, and possible mechanisms of the toxicity of doxorubicin were postulated. Lin et al. (2009) characterized the metabolism disorders of hepatotoxicity induced by CCl_4, a well-known model compound for inducing chemical hepatic injury, in a Wistar rat model with a single dosage of 1 ml/kg (Lin et al. 2009). The current metabonomic approach based on LC/MS indicated 23 endogenous metabolites as biomarkers in urine associated with the hepatotoxicity induced by CCl_4. The underlying regulations of CCl_4-perturbed metabolic pathways were discussed according to the identified metabolites. It suggested that the metabonomics is a potentially powerful tool for the discovery of drug safety biomarkers.

Since April 11 2001, FDA warns consumers to discontinue use of botanical products that contain *aristolochic acid*. Twenty-fifth European Directive 2000/11/EEC, specified that *aristolochic acid* and its salts, as well as *Aristolochia spp.* and their preparations are substances that act as powerful carcinogens. This are also prohibited in cosmetic products. Liang et al. (2009) performed a metabonomic method to investigate liver toxicity in rat urine after treatment of *Aristolochia fangchi* decoction. Along with the lasting of administration to four weeks, the renal injury in the *Aristolochia fangchi* group became more serious, and the contents of blood urea nitrogen and serum creatinine were all significantly higher as compared with the normal control group ($P < 0.05$). *Aristolochia fangchi* can induce renal lesions and its seriousness is correspondent to the lasting of administration. Prolonged exposure to aristolochic acid has shown to pose rapid progressive renal fibrosis in Belgium women in a slimming regimen in the early 90 s. The changes in metabolic profile could occurre before symptoms were observed, which may allow early treatment. Significant changes of two metabolite markers, kynurenic acid and hippuric acid, were detected and may act as preclinical markers for aristolochic acid exposure before symptoms observed (Chan et al. 2008). Urinary metabolic perturbations associated with liver toxicity induced by Huang-yao-zi (*root of Dioscorea bulifera L.*) were investigated using NMR to discover biomarkers for liver toxicity (Liu et al. 2010). The results revealed that the levels of taurine, creatine, betaine, dimethylglycine, acetate, glycine were elevated, whereas, the levels of succinate, 2-oxoglutarate, citrate, hippurate and urea were reduced. PLS-DA of NMR spectra revealed two apparent clusters between control groups and treatment groups, indicating metabolic changes observed in urine samples in response to Huang-yao-zi treatment. In addition, mechanism associated with hepatic injury was investigated. Metabonomics analysis in urine samples may be useful for predicting hepatotoxicity induced by Huang-yao-zi. A combined GC/MS and LC/MS metabolic profiling strategy indicates that *Tripterygium wilfordii Hook.* caused a time-dependent toxic effect at a high dose as revealed by the perturbed metabolic regulatory network involving disorders in energy metabolism, elevated amino acid and choline metabolism pathways, as well as altered structure of gut flora (Chen et al. 2008). The toxicological effects induced by realgar were investigated using metabonomic analysis (Wei et al. 2008). Signs of impairment of amino acid metabolism were supported by increased hepatic glutamate levels, increased methionine and decreased alanine levels in serum. The observed increase in glutathione in liver tissue aqueous extracts could be a biomarker of realgar induced oxidative injury. It illustrated the high reliability of NMR-based metabonomic approach on the study of the biochemical effects induced by traditional Chinese medicine. Wang et al. (2008) applied metabonomics to carry out characters of the hepatotoxicity induced by alcohol and the intervention effects of Yin Chen Hao Tang (YCHT), a classic TCM formula for treatment of jaundice and liver disorders in China. Urinary samples from control, alcohol- and YCHT-treated rats were analyzed by UPLC-MS in positive ionization mode. The total ion chromatograms obtained from the control, alcohol- and YCHT-treated rats were easily distinguishable using a multivariate statistical analysis method such as the PCA. The greatest difference in metabolic profiling was observed from alcohol-treated rats compared with the control and YCHT-treated rats. The positive ions m/z 664.3126 was elevated in urine of alcohol-treated rats, whereas, ions m/z 155.3547 and 708.2932 were at a lower concentration compared with that in urine of control rats, however, these ions did not indicate a statistical difference between control rats and YCHT-treated rats. The ion m/z 664.3126 was found to correspond to ceramide, providing further support for an involvement of the sphingomyelin signaling pathway in alcohol hepatotoxicity and the intervention effects of YCHT. It suggested that the metabonomics holds considerable promise and potential to significantly advance our understanding of the mechanistic bases for TCM.

4. Metabolomic biomarkers of nephrotoxicity

Drug-induced nephrotoxicity is a major concern, since many pharmacological compounds are filtered through the kidneys for excretion into urine (Li et al. 2011). Although early detection of toxicant induced kidney injury during drug development and chemical safety testing is still limited by the lack of sensitive and reliable biomarkers of nephrotoxicity, metabonomic technologies have brought enormous opportunities for improved detection of toxicity and biomarker discovery and made it more effectively and specifically. Changes in the concentration profiles of a number of small molecule metabolites found in either blood or urine can be used to localize kidney damage, assess organs at risk of rejection, assess kidneys suffering from injury or identify organs that have been damaged by drugs. Nevertheless, there are a number of easily measured metabolites in both urine and serum that can provide reliable indications of kidney function, kidney injury and drug toxicity (Isoda et al. 2011). Metabonomic experiments were performed to discover useful metabolites, whereas these metabolites are potential biomarkers for the early detection of drug-induced nephrotoxicity. Major metabolic pathways can be monitored by specific surrogate biomarkers in urine and blood using modern metabonomics technologies. Thus, this panel of biomarkers may provide a noninvasive method to detect kidney injury long before the onset of histopathological kidney damage. Metabonomic approach is a promising methodology for the rapid *in vivo* screening of nephrotoxicity associated with ingesting multi-ingredient medicinal herb supplements, and provides a valid method for comprehending the chemical-induced perturbations in the metabolic network and the networked lesions (Li et al. 2011).

For early detection of toxicity and obtained mechanistic information of toxicity, GC/MS-, NMR-, and LC/MS-based metabonomics by PCA and OPLS-DA were applied to urine samples from a rodent toxicity study on the mycotoxin and renal carcinogen ochratoxin A (Sieber et al. 2009a). Increased urinary glucose was a well-established indicator of kidney damage, and altered excretion of TCA cycle intermediates (citrate and 2-oxoglutarate) was found as a general response to toxic insult in many metabonomics studies. Markers are associated with cell proliferation (pseudouridine), changes in renal osmolyte handling (myo-inositol), and oxidative stress (5-oxoproline), established mechanisms of carcinogen ochratoxin A toxicity. Recent outbreak of renal failure in infants in China has been

determined to be caused by melamine and derivatives adulterated in the food. Metabonomics was performed to evaluate the global biochemical alteration triggered by melamine ingestion in parallel with the acute renal toxicity in rats (Xie et al. 2010). Urinary metabonomic profiles indicated that melamine resulted greatest renal toxicity in disrupted amino acid metabolism including tryptophan, polyamine, and tyrosine metabolism, and altered TCA and gut microflora structure. To evaluate untargeted metabolic profiling as a tool for gaining insight into the underlying toxicology, Fischer 344 male rats were dosed with 300 mg/kg/day of fenofibrate for 14 days and the urine and plasma were analyzed (Ohta et al. 2009). Reductions in TCA cycle intermediates and biochemical evidence of lactic acidosis demonstrated that energy metabolism homeostasis was altered. Perturbation of the glutathione biosynthesis and elevation of oxidative stress markers were observed. Furthermore, tryptophan metabolism was up-regulated, resulting in accumulation of tryptophan metabolites associated with reactive oxygen species generation, suggesting the possibility of oxidative stress as a mechanism of nongenotoxic carcinogenesis.

Sieber et al. (2009b) combined NMR and GC/MS metabonomics approach to assess the early detection of nephrotoxicity following treatment of male Wistar rats with gentamicin for 7 days. Altered excretion of urinary protein biomarkers detected Kim-1 and lipocalin-2, but not Timp-1 and clusterin, provides further support for lipocalin-2 and Kim-1 as sensitive, noninvasive biomarkers of nephrotoxicity. In addition, NMR-based metabonomics with multivariate statistics for monitoring biomarkers produced by doxorubicin treatments was carried out by Park et al. (2009). Urine samples from rats treated with doxorubicin at two dose levels were collected at each time point and doxorubicin-induced biomarkers were examined. Urinary increases in glucose, lactate, alanine, and valine suggested progression of renal damage resulting in glycosuria, lactic aciduria, and aminoaciduria up to 168 h in the high-dose group. Urinary elevation of creatine and phenylacetylglycine together with reduction of N-methylnicotinic acid and hippurate levels was suggestive of liver injury in the high-dose group. Impairment of energy metabolism was also indicated by decreased levels of tricarboxylic acid cycle intermediates in urine of rats treated with high-dose doxorubicin. The clinical use of the immunosuppressant calcineurin inhibitor cyclosporine is limited by its nephrotoxicity. A biomarker, 15-F(2t)-isoprostane, is identified for the development of toxicodynamic monitoring strategies and more detailed mechanistic insights for immunosuppressant nephrotoxicity (Klawitter et al. 2009). Results indicated that cyclosporine and/or sirolimus induce damage of the renal tubular system. This is reflected by urine metabolite patterns, which seem to be more sensitive than currently used clinical kidney function markers such as creatinine concentrations in serum. Metabonomics can provide proof of concept for further development of molecular marker strategy into diagnostic tools for the detection and monitoring of drug nephrotoxicity. Changes in urine metabolite patterns as a molecular marker are sufficiently sensitive for the detection of the ciclosporin's nephrotoxicity (Klawitter et al. 2010). The major kidney function markers were citrate, hippurate, lactate, TMAO, creatinine and phenylalanine. HPLC/MS-based metabonomic analysis was used to investigate urinary metabolic perturbations concerning the mechanism associated with D-serine-induced nephrotoxicity (Williams et al. 2005). D-Serine causes selective necrosis of the proximal straight tubules in the rat kidney accompanied by aminoaciduria, proteinuria and glucosuria. A general aminoaciduria, including proline, methionine, leucine, tyrosine and valine was also observed as well as an increase in acetyl carnitine. Investigation of additional metabolites altered as a result of exposure to D-serine is on-going. The present study proves the great potential metabonomics in mapping metabolic response for toxicology. *Morning Glory Seed* is a valuable traditional Chinese medicine which is widely used for the treatment of edema, ascites, hydroncus, simple obesity, lung fever and ardent fever. In recent years, long-term exposure to *Morning Glory Seed* has shown to pose progressive renal damage in clinical practice (Ma et al. 2010a, b). Recently, an UPLC/MS metabonomic approach was employed to characterize the comprehensive metabolic syndromes of MGS-induced nephrotoxicity. Significant changes of 12 metabolite biomarkers were detected in the rat urine samples (Ma et al. 2010c). The results indicated that certain metabolic pathways, such as lysophosphatidylcholines formation and sphingolipids cycle were accelerated, while the phenylalanine level in serum was decreased. Metabonomic approach is helpful to further understanding and clinical diagnosis of TCM induced nephrotoxicity. In the future, we expect to expand the potential biomarkers of hepatotoxicity and nephrotoxocity to verify in the clinic.

5. Conclusion and future perspectives

Metabonomics has shown the potential to enable mapping of early biochemical changes in toxicity and hence exerts an opportunity to develop predictive biomarkers that can trigger earlier interventions as well as could provide valuable insights about mechanisms of toxicity. As a systems toxicology approach, it was able to provide comprehensive information on the dynamic process of drug toxicity. Application of metabonomics technology during drug discovery and development is rapidly evolving. In this review, we delineate and evaluate the current status of metabonomics in the field of drug-induced hepatotoxicity and nephrotoxicity, with an emphasis on metabolic biomarker discovery. New novel and specific biomarkers of drug toxicity could contribute and facilitate the development of pharmaceutical companies, thus benefiting the public and improving the drug development paradigm. Many of these markers have been confirmed across multiple studies and can earlier detect toxicity than the traditional clinical chemistry and histopathology methods. Upon further validation, such markers will offer clear benefits for the pharmaceutical industry and regulatory agencies. Metabonomic technologies have the capability of providing diagnostic and prognostic biomarkers specific for early stages of liver and kidney injury, help to define patterns of toxicity for early identification of potential adverse effects for drug, advancing our understanding of the mechanistic bases for drug safety assessment. The significance of recent advancements in the application of metabonomic analyses in the assessment of drug toxicity is highlighted once more. In the near future, a strong understanding of metabonomics and its potential uses for discovering novel and non-invasive biomarkers at early stages of toxicity will be critical to the drug development process and clinical practice.

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