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The Opportunities of Metabolomics in Drug Safety Evaluation

Pengcheng Wang, Amina I. Shehu, and Xiaochao Ma*

Center for Pharmacogenetics, Department of Pharmaceutical Sciences, School of Pharmacy, University of Pittsburgh, Pittsburgh, PA, USA

Abstract

Although safety of drug candidates is carefully monitored in preclinical and clinical studies using a variety of approaches, drug toxicity may still occur in clinical practice. Therefore, novel approaches are needed to complement the current drug safety evaluation system. Metabolomics comprehensively analyzes the metabolites altered by drug exposure, which can therefore be used to profile drug metabolism, endobiotic metabolism, and drug-microbiota interactions. The information from metabolomic analysis can be used to determine the off-targets of a drug candidate, and thus provide a mechanistic understanding of drug toxicity. We herein discuss the opportunities of metabolomics in drug safety evaluation.

Keywords

Metabolomics; drug metabolites; endobiotic metabolites; drug safety evaluation

Introduction

Despite great efforts and progress having been made in the field of drug safety evaluation, adverse drug reactions (ADRs) remain a major concern in the clinic [1, 2]. The incidence of serious ADRs was 6.7% of hospitalized patients, out of which 0.32% are fatal ADRs [3]. The management of ADRs is a great burden for both patients and health providers [4]. Besides, drug toxicity remains a major cause of drug development failure during preclinical and clinical studies [1]. ADRs caused by a few drugs like acetaminophen (APAP) are dose-dependent and they can be predicted. However, many ADRs are idiosyncratic which makes them difficult to predict [5].

Drug safety evaluation is an important component of drug development and also a top priority for regulatory agencies. Safety evaluation is required across the whole process of drug development, from preclinical studies to clinical trials, as well as post-approval safety surveillance. Biochemical and histological analysis are the major approaches used for drug safety evaluation. These approaches are effective in most cases to determine the safety profile of drug candidates. However, limitations of these biochemical and histological

*To whom correspondence should be addressed: 309 Salk Pavilion, 335 Sutherland Drive, Pittsburgh, PA 15261. Phone: 412-648-9448. mxiaocha@pitt.edu.

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approaches are obvious: (1) these methods have fallen short in preventing unsafe molecules from getting into the market, especially for toxicities that often originate from off-target effects; and (2) these methods cannot provide detailed information to identify unexpected targets and explore the mechanism of drug toxicity [6, 7]. Therefore, new strategies are needed for drug safety evaluation. Modern technologies have been adopted in the research field of drug toxicity, which include genomics, proteomics, transcriptomics and metabolomics [5, 8, 9]. There are excellent reviews focused on the applications of genomics, proteomics, and transcriptomics in drug toxicity studies [5, 10-12]. This review will focus on the applications of metabolomics in drug safety research and evaluation.

Metabolomics is the comprehensive and quantitative analysis of all metabolites within a biological system [7, 13-15]. It illustrates the alteration of terminal metabolites and biochemical basis in response to stress [14]. Metabolomics has been successfully used for investigating toxicities of multiple drugs and xenobiotics (Table 1). The current review highlighted the power of metabolomics in profiling drug metabolism and bioactivation pathways, endobiotic homeostasis, drug-endobiotic interactions, and discussed their associations with drug toxicity.

Metabolomics for screening drug metabolism and bioactivation pathways

Most drugs undergo biotransformation once they enter the body. Based on the pharmacological and toxicological properties of drug metabolites, they can be classified into three types: active metabolites, inactive metabolites, and reactive metabolites [36]. The reactive metabolites can bind to cellular macromolecules, such as proteins and DNA, disrupt normal cellular functions, and lead to toxicity [37-39]. Therefore, screening of reactive metabolites is needed in the early stage of drug development [38-41]. Since reactive metabolites are unstable and sometimes unpredictable, they are hard to be detected and identified.

Metabolomics provides an efficient tool to profile drug metabolism and bioactivation, especially for the reactive and unexpected metabolites. The LC-MS-based metabolomic approach has been successfully used to profile reactive metabolites of pulegone (PLG), a hepatotoxin from a variety of plants [42, 43]. Glutathione (GSH) and semicarbazide were used to trap the reactive metabolites of PLG in human liver microsomes [37]. The principal component analysis (PCA) and the orthogonal partial least squares-discriminant analysis (OPLS-DA) conveniently identified PLG-GSH conjugates and PLG-hydrozones. Besides, PLG-pyridazine, an unpredicted adduct, was identified from the metabolomic analysis [37]. The LC-MS-based metabolomic approach has also been used to screen the bioactivation pathways of ritonavir (RTV) [44]. RTV is a critical component of protease inhibitor-based regimens for antiretroviral therapies [45]. RTV-containing regimens frequently cause liver injury [46-49]. Metabolomic analysis revealed twenty-six metabolites including five reactive metabolites that may be associated with RTV hepatotoxicity [44]. These findings suggest that metabolomics is a powerful and straightforward technical platform for investigating drug metabolism and bioactivation.

Metabolomics for profiling the effects of drugs on endobiotics homeostasis

Chemical insults may affect the equilibrium of endogenous metabolites in an organism because of on-target or off-target effects [50]. Metabolomics can recapture biochemical alterations of endobiotics and thus uncover drug targets. APAP is a widely used over-the-counter analgesic and antipyretic drug. APAP overdose causes serious liver injury, which accounts for nearly half of all cases of acute liver failure in the United States [51]. Depletion of endogenous antioxidant glutathione (GSH) and mitochondrial injury have been identified as critical steps in APAP hepatotoxicity [52]. A metabolomic approach was used to revisit the mechanisms of APAP hepatotoxicity, which found that GSH is the number one decreased ion in the liver of mice treated with APAP [37]. Metabolomics study also revealed the accumulation of acylcarnitines in the serum of mice treated with APAP, suggesting that APAP disrupts fatty acid oxidation in mitochondria [25, 53].

Both rifampicin (RIF) and isoniazid (INH) are the first-line anti-tuberculosis drugs. Combined therapies with RIF and INH can cause liver injury and even liver failure [54]. Previous studies proposed that reactive metabolites and oxidative stress were associated with the hepatotoxicity of RIF and INH [55, 56], but the detailed mechanisms remain unknown [57]. A recent metabolomics study provided a novel insight into RIF and INH-induced liver injury through the interactions with endobiotics [58]. Since RIF is a human specific pregnane X receptor (PXR) ligand, PXR-humanized mice were used to investigate the hepatotoxicity associated with RIF and INH. Significant elevation of liver injury was only observed in PXR-humanized mice co-treated with RIF and INH, but not in wild-type mice and *Pxr*-null mice, suggesting that human PXR is the key modulator of liver injury in RIF and INH co-therapy [58]. Analysis of bile metabolome showed that co-treatment with RIF and INH in PXR-humanized mice caused a significant accumulation of protoporphyrin IX (PPIX), an intermediate in the heme biosynthesis pathway that can cause hepatocellular and cholestatic liver injury [59, 60]. Further studies found that both RIF and INH target on the heme biosynthesis pathways and lead to PPIX accumulation [58, 61].

Lipidomics focuses on measuring the alterations of cellular lipids on a large scale [62], which therefore provides an ideal tool to explore the effects of drug exposure on cellular lipid homeostasis. Inhibition of mitochondrial fatty acid oxidation and disruption of cellular lipid metabolism are associated with drug hepatotoxicity [63, 64]. Cocaine is a widely abused psychological stimulant that can cause liver injury in humans and mice [65, 66]. Lipidomics has been used to elucidate the mechanism of cocaine hepatotoxicity [67]. Treatment with cocaine in mice resulted in an accumulation of long-chain acylcarnitines in the liver, suggesting that cocaine can inhibit mitochondrial fatty acid oxidation [67]. This idea is supported by the fact that fenofibrate, a PPAR α activator, increases mitochondrial fatty acid oxidation and attenuates cocaine-induced liver injury [67].

The three cases discussed above illustrate the capability of metabolomics in identifying off-targets of drugs through profiling endogenous metabolites. This strategy may provide a novel avenue to investigate idiosyncratic ADRs. Idiosyncratic ADRs are usually not observed in clinical trials until a large population are exposed to the drug [68]. Individual differences in drug metabolism and immune response have been proposed as major

mechanisms of idiosyncratic ADRs [69-72]. However, most cases of idiosyncratic ADRs cannot be explained by individual differences in drug metabolism and immune response. Therefore, novel concepts and techniques are needed to illustrate the mechanisms of idiosyncratic ADRs. For idiosyncratic ADRs, multiple risk factors have been found in clinical studies [50, 73, 74]. It is possible that a drug disturbs certain metabolic pathways of endobiotics in the body, and that the cofactors that double hit on these metabolic pathways will potentiate drug toxicity. Untargeted metabolomics provides an ideal tool to identify both drug-specific and patient-specific alterations of endobiotics, and thus may facilitate a mechanistic understanding of idiosyncratic ADRs.

Metabolomics for profiling drug-microbiota interactions

Aside from profiling drug-endobiotic interactions, metabolomics has been used to study drug-microbiota interactions [26, 75, 76]. Emerging evidences suggest that gut microbiome play critical roles in drug metabolism and toxicity [77-79]. Metabolomic analysis has found that *p*-cresol, a gut microbiota product, can affect APAP hepatotoxicity [26]. Further studies revealed that *p*-cresol is a substrate of sulfotransferase 1A1 (SULT1A1), which is also responsible for *O*-sulfonation of APAP [26]. Therefore, *p*-cresol competes with the detoxification pathway of APAP and potentiates APAP hepatotoxicity.

Summary and perspectives

After drug exposure, the major changes in metabolome are: (1) the accumulation of the drug and its metabolites; and (2) the alteration of a host's endobiotics and metabolites of microbiota in response to a drug and its metabolites. Metabolomics can profile drug metabolism, drug-endobiotic interactions, and drug-microbiota interactions (Figure 1). The information obtained from metabolomic analysis can be used to determine the off-targets of a drug candidate, and thus provide a mechanistic understanding of drug toxicity. In summary, metabolomics has great opportunities to complement the current drug safety evaluation system.

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Figure 1. The opportunities of metabolomics in drug safety evaluation

Metabolomics can systematically profile drug metabolism, endobiotic metabolism, and drug-microbiota interactions. The information from metabolomic analysis can be used to illustrate drug targets and provide a mechanistic understanding of drug toxicity. The following analyses are recommended for use of metabolomics in drug safety evaluation: (1) metabolomic analysis of blood, urine, and feces from preclinical to clinical studies; and (2) metabolomic analysis of major organs in preclinical studies.

Table 1

Representative studies on drug and xenobiotic toxicity using metabolomics.

Drugs and xenobiotics	Biological specimens	Technological platform	References
Ammonia, dimethylsulfoxide, APAP	Bioartificial organs	NMR	[16]
Teratogens	Human embryonic stem cells	LC-MS	[17, 18]
Flutamide, hydroxyflutamide	HepG2/C3a cells	NMR	[19]
Amiodarone, clozapine, cyclosporine, doxycycline, fluoxetine, tamoxifen, tert-Butyl hydroperoxide, tetracycline, tilorone, valproic acid, ketotifen, cumene hydroperoxide	HepG2 cells	LC-MS	[20, 21]
Nonylphenol	Rat urine	GC-MS	[22]
Carbon tetrachloride, APAP, methotrexate	Rat urine	LC-MS	[23]
APAP	Mouse urine and serum; human using and serum	LC-MS, NMR	[24-27]
A wide range of model toxins including hydrazine	Rat urine and serum	NMR	[28]
Green tea extract, APAP	Mouse liver	LC-MS, NMR	[29]
MrkA	Rat urine and liver	NMR	[30]
Carbon tetrachloride, α -naphthylisothiocyanate, 2-bromoethylamine, 4-aminophenol	Rat serum	NMR	[31]
Gentamicin	Rat urine	NMR, LC-MS	[32]
Hydrazine	Rat urine	NMR	[33]
Griseofulvin	Mouse liver	LC-MS	[34]
Doxorubicin, isoproterenol, 5-fluorouracil	Rat plasma	LC-MS	[35]

GC, gas chromatography; LC, liquid chromatography; NMR, nuclear magnetic resonance spectroscopy; MS, mass spectrometry.