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REVIEW ARTICLE

Next-generation Metabolomics in the Development of New Antidepressants: Using Albiflorin as an Example

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Abstract: Depression is a highly prevalent disorder that affects more than 300 million adults worldwide in 2015. Depression also frequently coexists with many other conditions such as osteoporosis and one-third of the Intensive Care Unit (ICU) survivors had depressive symptoms. Antidepressants have become the most commonly prescribed drugs in the United States. In addition to the regular process, drug discovery and development (R&D) for depression presents extra challenges because of the heterogeneity of the symptoms and various co-occurring disorders. Botanical medicine with multi-functional nature has been proposed to be more effective, providing rapid control of core and comorbid conditions of depression. With the technical advances in analytical instruments, metabolomics is entering into a "new generation". Next-generation metabolomics (NGM) has the capability to comprehensively characterize drug-induced metabolic changes in the biological systems. NGM has demonstrated great potential in all the stages of pharmaceutical R&D in the last 10 years. Albiflorin isolated from Peony roots is a promising drug candidate with multi-target for depression and is currently under development by Beijing Wonner Biotech. In this work, we summarized the common analytical platforms for NGM and its main applications in drug R&D. We used albiflorin as an example to illustrate how NGM improves our understanding of drug candidate actions and facilitates drug safety evaluation. Future directions on how to expand the use of NGM for new antidepressant development in pharmaceutical industry were also discussed.

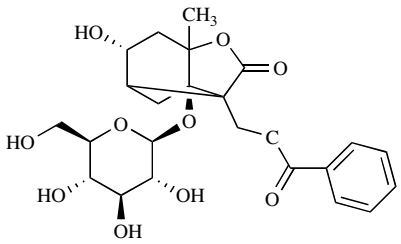
Keywords: Metabolomics, antidepressants, drug development and discovery, albiflorin, depression, next-generation metabolomics (NGM).

1. INTRODUCTION

Metabolomics is the new "omics" technology added to the system biology toolbox in the post-genomic era [1]. Metabolomics uses various advanced analytical chemistry approaches to characterize the metabolites and metabolism in biological systems. Metabolites are the direct "readout" of gene-environment interactions (G x E = metabolism). Unlike genomics, transcriptomics, and proteomics that can only tell us what might happen, metabolomics reflects what is currently happening. Metabolomics has broad applications in many diverse areas such as diseases pathogenesis and diagnosis, biomarker screening, drug discovery, precision medicine, plant biology, microbiology, nutrition and environmental monitoring [2-7].

Depression is one of the most common diseases and the leading cause of disability. It was reported that depression affects at least 4.4% of the world population in 2015 [8]. Depression is a multifactorial disorder with the abnormalities of cognitive functions, sleep and gut microbiome [9]. The development of drugs for major depressive disorder is particularly challenging due to the complexity of the symptoms, heterogeneity and comorbid diagnosis. The major classes of approved antidepressants include Selective Serotonin Reuptake Inhibitors (SSRIs), Tricyclic Antidepressants (TCAs) and monoamine oxidase inhibitors (MAOIs) [10]. However, current antidepressants are limited by their undesirable side effects and slow onset [11]. In addition, nearly 50% of patients do not respond to the first medication prescribed [12]. There is an urgent need for the development of new antidepressants.

Medicinal plants are rich reservoirs for new drugs and drug leads. Over the past decade, several medicinal plants and formulations have been used for the treatment of depression such as turmeric (*Curcuma longa*), St John's wort (*Hypericum perforatum*), white peony (*Paeonia lactiflora* Pall.) and lavender (*Lavandula* spp.) [13]. The dry root of white peony has been used as herbal medicine in China, Korea, and Japan for the treatment of major depressive disorder for hundreds of years [14]. Albiflorin is one of the main bioactive molecules isolated from the roots of white peony (Fig. **1**). Recently, albiflorin was shown to be a promising new antidepressant [15]. Unlike the highly selective single target antidepressants, albiflorin is a novel serotonin (5-HT) and norepinephrine (NE) reuptake inhibitor in the hippocampus [16, 17]. The dopaminergic system also contributes to albiflorin-mediated antidepressant-like activity [17]. Given the heterogeneous nature of depression, multi-target agents like albiflorin have been proposed to be more effective to simultaneously control core and comorbidities of depression than the classical antidepressants with highly specific and selective single target on neurotransmitters [18].



Albifloria

Fig. (1). Chemical structure of albiflorin isolated from the roots of *Paeonia lactiflora* Pall.

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The use of metabolomics in drug discovery and development is also called pharmacometabolomics. The traditional drug discovery and development process are costly and time-consuming. A standard process takes more than 10 years from the beginning to the market and costs over $800 million with the success rate as low as 7% [19] (Fig. **2**). Pharmacometabolomics dramatically facilitates this process and reduces the overall cost. It can be useful in all stages of drug discovery and development, providing holistic information for the determination of drug targets, models of actions, safety and toxicity evaluation (Fig. **2**). Pharmacometabolomics is particularly essential for drugs with multi-targets which even take longer time than the development of a single target drug using traditional drug discovery approach.

In this work, we summarized the role of metabolomics in the discovery and development of antidepressants, particularly albiflorin, in relation to novel antidepressants with multi-targets from medicinal plants. Since the use of metabolomics in target identification, chemical library screening and molecular mechanisms of drugs had been extensively discussed recently [1, 20, 21], here, we focused on its application in drug toxicity and safety evaluation.

2. NEXT-GENERATION METABOLOMICS (NGM)

The origins of metabolomics can be traced back to the newborn screening of inborn errors of metabolism in clinical chemistry since the 1960s, which was much further than genomics and proteomics [22]. The term "metabolome" was coined in 1998 by Steven Oliver in a review article [23]. The first metabolomics paper was published by the Nicholson group in 1999 [24]. In the first ten years of 21 century (2000-2010), the early applications of metabolomics were primarily conducted using ^{1}H nuclear magnetic resonance spectroscopy (^{1}H NMR) and gas chromatography coupled to mass spectrometry (GC-MS) [25, 26]. However, metabolite measurements using NMR and GC-MS are restrained by the chemical diversity of metabolites and their broad dynamic range in cellular abundance [27]. NMR has relatively low sensitivity (limit of detection >5 μM) and cannot detect non-protonated metabolites [28]. GC-MS can only detect volatile compounds and require sophisticated sample derivatization steps for other non-volatile metabolites [4, 29]. In addition, lack of fragmentation information by GC-MS makes the identification of metabolites less accurate [4]. In the last 10 years (2008-now), with the advancements of separation and mass spectrometry detection techniques, NGM was developed on several different platforms [4, 30, 31]. These platforms provide a broad coverage of different classes of metabolites, superior detection sensitivity, high resolution and mass accuracy [31-35]. Single-cell metabolomics and imaging metabolomics were also developed in recent years [36, 37].

The brief workflow for NGM is shown in Fig. **3**. A careful experimental design is essential for the success of a metabolomic study. Enough biological replicates should be prepared in order to draw the reliable conclusion. For human subjects, at least 15 subjects should be recruited for each group and gender. Age and gender should be matched between controls and diseases (treatment). The minimum sample size for animals and plants is 8 per group. The minimum sample size for *in vitro* cells is 6 per group. For cells, it usually requires at least one million cells depending on the sensitivity of the instruments. Metabolites are extracted from the samples using different extraction methods including organic solvents deproteination and liquid-liquid extraction [38]. Metabolites are then separated and detected. The peaks are checked and statistical analysis is conducted.

Table **1** and Fig. **4** summarize the applications of different platforms for NGM. Due to the high chemical diversity of the metabolites and the limited scan speed for the detectors, separation is usually required for metabolomics. GC, Liquid Chromatography (LC) and Capillary Electrophoresis (CE) are the common separation techniques used in NGM [39]. Occasionally, two-dimensional separation by GC×GC or LC×LC was required [40]. In general, GC is a method of choice for nonpolar or volatile and thermal stable metabolites such as fatty acids. Polar metabolites can also be analyzed by GC after derivatization [41]. LC is the most versatile separation

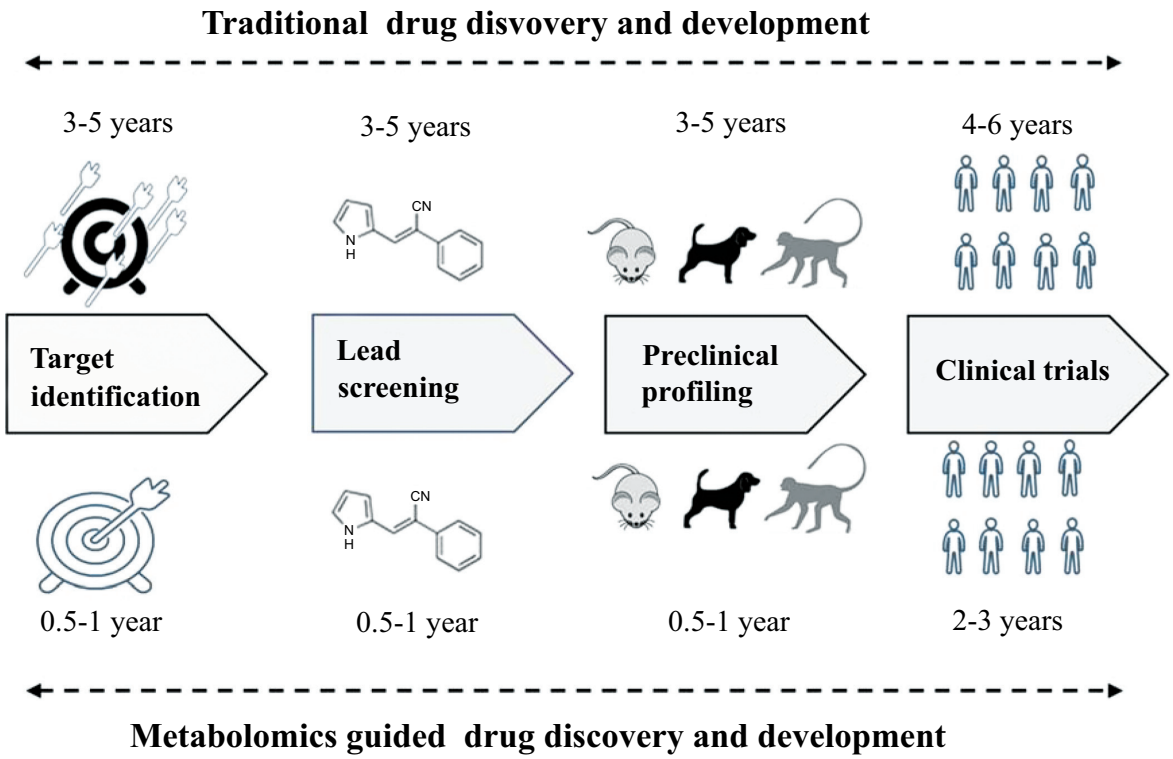


Fig. (2). Metabolomics facilitates all the stages of drug discovery and development.

method, allowing the separation of compounds of a wide range of polarity [42]. HILIC phase is useful for the analysis of polar metabolites such as amino acids, organic acids, and sugars, while, nonpolar and weak polar metabolites can be separated by reverse phase columns (C18, biphenyl, *etc*.). Derivatization is generally not required for LC analysis. CE has high separation efficiency for polar ionic metabolites such as carbohydrates and amino acids. In addition, CE is especially suitable for volume-restricted biological samples such as spinal fluid and single cell extract [35, 43].

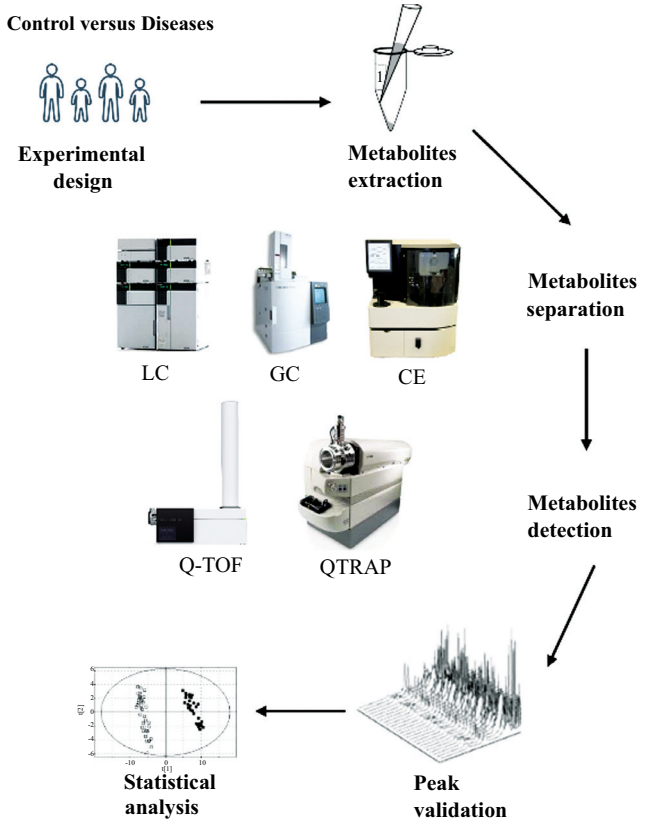


Fig. (3). The schematic representation of next-generation metabolomics workflow.

Mass Spectrometry (MS) is the most common detection approach for NGM due to its high sensitivity, selectivity and potential to identify the metabolites [44]. In the last few years, various MS analyzers were commercialized by different vendors including the time of flight MS (TOF/MS, LecoCorp), quadrupole TOF (Q-TOF, Agilent), triple quadrupole (MS/MS, SCIEX, Shimadzu, and Waters) and orbitrap (Thermo) [45]. High-resolution TOF instruments coupled to GC, LC or CE are often used for untargeted metabolomics, while, triple quadrupole (QqQ) instruments are applied for targeted metabolomic analysis.

Metabolomics at the single-cell level is essential for the analysis of phenotypic heterogeneity between cells such as neurons in which individual differences are required for function [63]. The cells are sampled by fine-tip glass capillaries, Laser Capture Microdissection (LCM), optical trapping, Fluorescence-Activated Cell Sorting (FACS) and Magnetic-Activated Cell Sorting (MACS) depending on the cell types [64]. Due to the limited volume size for single cell extract, CE and nano-flow LC are the main front-end separation techniques for single cell metabolomics [65, 66]. MS is the major technique for detection as other metabolomics.

Imaging metabolomics is also a powerful tool to explore the model of actions of drugs, especially for drug candidates with multitarget [37]. Several well-developed mass spectrometry imaging (MSI) techniques are currently in use for the performance of imaging metabolomics such as high-resolution matrix-assisted laser desorption ionization (MALDI)-MSI [67, 68], TOF-secondary ionization mass spectrometry (TOF-SIMS) [69], desorption electrospray ionization (DESI)-MSI under ambient conditions [70, 71] and air-flow-assisted desorption electrospray ionization [72] (Fig. **4**). Both molecular and spatial information can be obtained simultaneously by imaging metabolomics.

3. METABOLOMICS IN EXPLORING THE MECHANISM OF ACTION (MOA) OF ANTIDEPRESSANTS

To minimize the problems in downstream steps such as preclinical and clinical trials, it is desirable that MOA of any drug candidates is established during the drug discovery process [73]. Depression has a strong metabolic basis or a clear metabolic cause such as the metabolic perturbations in tryptophan, kynurenine and mitochondrial metabolism [74-76]. The discovery of the metabolic basis of depression often leads to the development of new therapeutic solutions. Metabolomics thus might provide a far more cost-effective and productive approach to elucidate MOA of antidepressants.

Some software and tools used for dissecting the MOA of drugs have been developed. Metaboanalyst (www.metaboanalyst.ca) and Metlin (https://metlin.scripps.edu) are free online tools for comprehensive metabolomic data analysis [77, 78]. Ingenuity Pathway Analysis (IPA)/KEGG pathway analysis is a licensed software for the integration of multi-omic data including metabolomics, proteomics and transcriptomics.

Recently, metabolomics has been successfully applied to identify the molecular mechanisms of drug or drug candidates with

Table 1. Next-generation metabolomics platforms.

Separation Techniques		MS (Vendors)	Main Applications	References
GC or GC×GC	DB-5MS (Agilent); RTX-5MS (Restek)	TOF/MS (LecoCorp); Q-TOF (Agilent); Orbitrap (Thermo); oa-TOF(Waters)	Fatty acids, volatiles; nonpolar; polar compounds (requires derivatization)	[46-50]
LC	HILIC (amino, amide, diol, etc)	MS/MS (triple quadrupole, SCIEX, Shimadzu, Waters); TripleTOF (SCIEX); Q-TOF (Agilent); Orbitrap (Thermo)	Polar metabolites such as amino acids, organic acids, and sugars	[51, 52]
	RPLC (C18, C8, Biphenyl, etc)		Nonpolar and weak polar metabolites such as phospholipids and ceramides	[53-56]
CE	Fused-silica capillaries	TOFMS (Agilent); Orbitrap (Thermo); microTOF (Bruker); MS/MS (SCIEX)	Polar and charged metabolites such as carbohydrates and amino acids	[57-62]

Abbreviations: HILIC: Hydrophilic interaction liquid chromatography; RPLC: reverse phase; MS: mass spectrometry; TOF: Time of flight

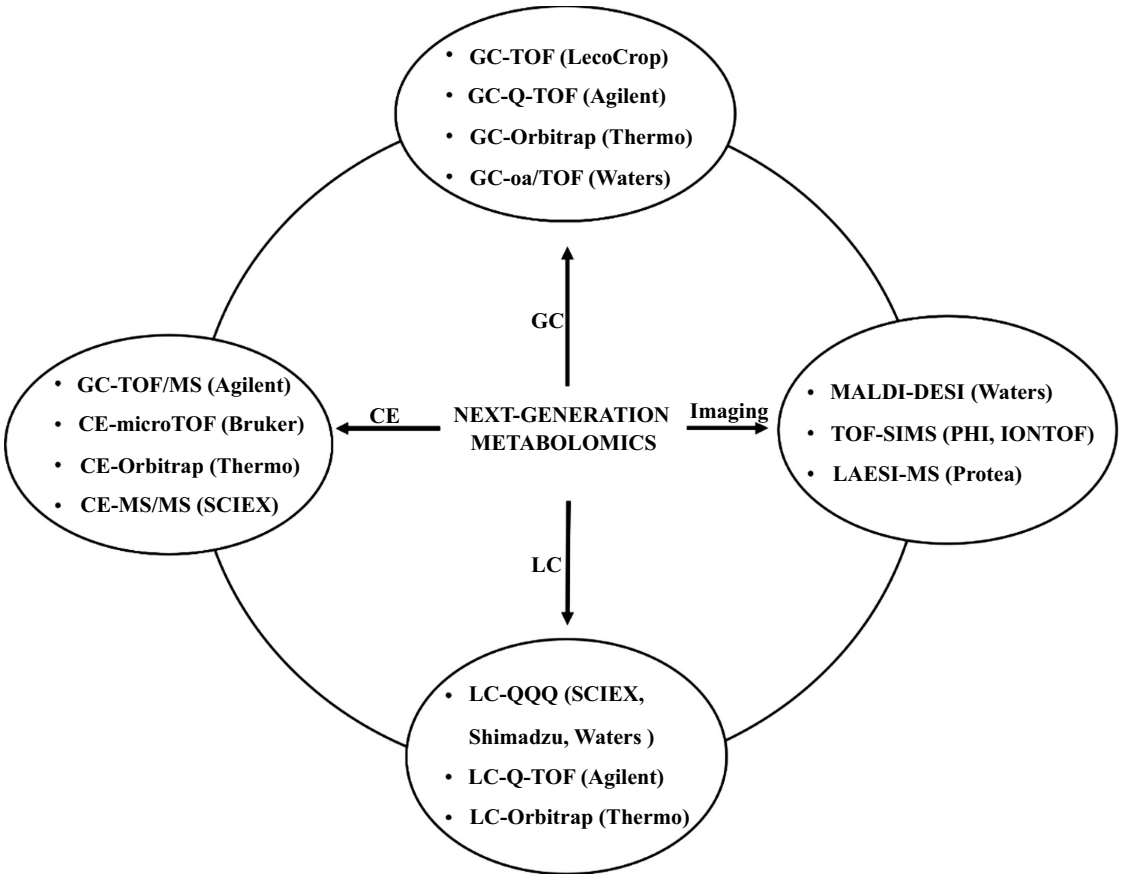


Fig. (4). The next-generation metabolomics platforms. Gas chromatography: GC; Capillary electrophoresis: CE; Liquid chromatography: LC; Mass spectrometry: MS; Time of flight: TOF; Triple quadrupole: QQQ; matrix-assisted laser desorption ionization: MALDI; TOF-secondary ionization mass spectrometry: TOF-SIMIS; Desorption electrospray ionization: DESI; Laser ablation electrospray ionization: LAESI.

antidepressant activity. Ketamine is a promising fast-acting antidepressant. By GC-MS based metabolomics, Liang and the coauthors showed that ketamine exerts its antidepressant effects through an alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) inhibition-dependent mechanism [79, 80]. Metabolomics revealed that the antidepressant action of diterpene ginkgolides is mainly related to amino acid, energy and lipid metabolism [81, 82]. The mechanism of antidepressant activity of chlorogenic acid is found to be involved in regulating the abnormal pathways of nicotinate and nicotinamide metabolism [83]. In addition, the antidepressive effects of Yangxinshi Tablet [84], Chaihu-Shu-Gan-San [85], venlafaxine [86] and scopolamine [87] were investigated by NGM.

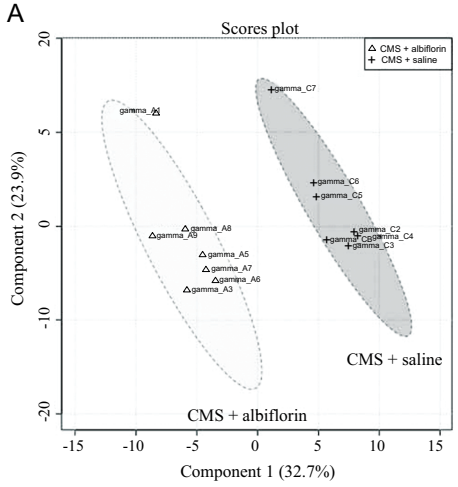
3.1. Case Study 1-Metabolomic Profiling of Antidepressant Drug Action of Albiflorin

Here, we used albiflorin to demonstrate how metabolomics-based drug discovery may be applied to elucidate MOA of antidepressants. The rat model of depression was generated through 4-week exposure to Chronic Mild Stress (CMS) to male Sprague Dawley (SD) rats. Rats with CMS were then treated with albiflorin (7 mg/kg/d) or saline for 7 days. Plasma samples were collected before and after albiflorin treatment. Using the next-generation broad-spectrum targeted metabolomics [88], we found that plasma metabolites displayed dramatic differences between CMS + albiflorin and CMS + saline (Fig. **5A**). We discovered a higher level of acetylcarnitine, 3-Hydroxydodecanoylcarnitine as well as myristoyl carnitine and a lower level of citric acid, succinic acid and creatine in the urine of rats exposed to CMS (Fig. **5B**). Pathway analysis showed that these changes of metabolites were involved in fatty acid beta-oxidation and citric acid cycle in mitochondria. The disturbed metabolic pathways were completely normalized by albiflorin treatment. Metabolomic analysis indicated that albiflorin might target on mitochondrial energy metabolism.

4. METABOLOMICS IN EVALUATING THE TOXICITY OF ANTIDEPRESSANTS

Although certain drugs can directly affect gene regulation, significant drug-induced effects are usually due to the changes of endogenous metabolites concentrations [89, 90]. Metabolite levels in the biological fluids are the readout of intra-tissue and intra-organ homeostasis [91]. The disturbances of metabolites by drugs lead to homeostasis imbalance. The consequences are recognized as drug toxicity. Metabolomics thus provides the information on the functional integrity of the whole organism over time after drug exposure. The main purpose of metabolomics is to quickly establish the toxicology and pharmacokinetics of compound candidates and reduce the drug failures that are due to toxicity in late-phase trials. The application of metabolomics for drug toxicity evaluation in the pharmaceutical industry started in the early 2000s [92, 93]. Studies before 2010 had been well summarized by several reviews written by the authors from both academics, US FDA and the leading pharmaceutical companies [92, 94, 95]. Perhaps, the greatest efforts were conducted by the Consortium for Metabonomic Toxicology (COMT), which was formed between five major pharmaceutical companies and academics from Imperial College London, UK [96]. Their work established a predictive expert system for predicting organ toxicity of drug candidates using metabolomics and nearly 150 studies were completed in 3 years [97]. The example of COMT clearly demonstrated that it is possible to use metabolomics to pro-

A



B

Metabolites	Log2(Albiflorin/saline)	p value
Succinic acid	3.1476	0.000171
L-Threonine	2.9506	6.72E-05
Citric acid	2.8406	1.06E-05
Creatine	2.3953	0.03534
Docosahexaenoic acid	1.9479	0.000581
Glucose	1.5812	0.000596
Acetylcholine	1.392	0.015337
S-Adenosylhomocysteine	-1.0808	0.016305
Acetylcarnitine	-1.2519	0.043744
3-Hydroxydodecanoylcarnitine	-1.2902	0.015337
Norepinephrine	-1.3081	0.00648
Uric acid	-1.338	0.016832
Myristoyl carnitine	-1.4503	0.006439
Oxaloacetic acid	-1.4519	0.016305

Fig. (5). Metabolomic analysis revealed that albiflorin might exert antidepressant-like activity through targeting on mitochondrial metabolism. **A**: OPLS-DA revealed the dramatic metabolites differences in urine between CMS rats with and without albiflorin treatment. **B**: Metabolites significantly altered after albiflorin treatment.

vide high throughput and inexpensive screening for large-scale drug toxicity assessment.

4.1. *In vivo* Toxicity Screening Models

In vivo drug toxicity evaluation using rodents (*i.e*., mice and rats) and non-rodents (*i.e*. dog, non-human primate) is used to assess organ toxicity, reproductive, developmental toxicity and genotoxicity and establish a safe starting dose for clinical studies [98]. Table **2** summarizes the published in vivo drug toxicity evaluation using metabolomics. Metabolomic profiling was showed to be more sensitive than the conventional techniques in the detection of potential drug hepatotoxicity in rats [99]. Griseofulvin is an antifungal medication used to treat a number of types of infections. Metabolomic analysis elucidated the biochemical basis of griseofulvin-induced liver injury, which was related to the significant accumulation of protoporphyrin IX (PPIX), N-methyl PPIX, bile acids, and glutathione (GSH) in the liver [100]. Using a targeted metabolomic approach, James, *et al*., found that glycodeoxycholic acid, taurodeoxycholic acid, and glycochenodeoxycholic acid were significantly elevated in the serum of children with acetaminophen overdose, indicating the injury of the liver [101]. Aranibar, et al., from Bristol-Myers Squibb laboratories identified ascorbic acid and gulonic acid in urine as the biomarkers of liver cytochrome P450 induction using NMR-based metabolomic profiling [102].

A metabolomics guided compound ranking system was developed to help the selection drug candidates with least toxic side effects on kidney in early drug development [103]. Urinary metabolomic profiling in combination with multivariate analysis led to the separation of post-dose time points and controls. Further analysis identified choline as a potential biomarker in urine for drug toxicity to the kidney. Doxorubicin is a chemotherapy medication widely used to treat different types of cancer such as breast cancer, bladder cancer and acute lymphocytic leukemia [104]. The systemic processes of doxorubicin-induced toxicity including onset and progression were elucidated using NGM. The increase of urinary uric acid, tryptophan, and phenylalanine and the decrease of hippuric acid and 2, 8-quinolinediol glucuronic acid were associated with the progression of doxorubicin-induced toxicity [105]. Tacrolimus is an immunosuppressant widely used to prevent rejection after the organ transplant. Its toxicity has to be carefully monitored during the treatment. Targeted metabolomics identified 3 potential human urinary biomarkers including glucose, sorbitol, and trimethylamine oxide (TAO) which can be used for monitoring the toxicity onset of tacrolimus to the kidney [106]. Later, researchers reported the utility of urinary 1 and 3-methylhistidine as putative biomarkers of cerivastatin-induced skeletal muscle necrosis and hypertrophy in the rats [107]. An increase in urinary excretion of 1- and 3-methylhistidine was associated with the rat skeletal muscle injury caused by cerivastatin administration.

Metabolomics has also been used to evaluate the toxicity of medicinal plants used in traditional medicine. Caowu (*Radix Aconiti kusnezoffii*) is a medicinal plant that has been widely used in the treatment of rheumatic arthritis, painful joints, and tumors in China. Yan and the co-authors investigated the potential liver and heart toxicity after long-term administration of Caowu by LC-MS/MS based metabolomics [108]. The urinary metabolomics revealed the severe toxicity to heart and liver induced by Caowu. Total 13 phenotypic toxicity biomarkers were identified in urine such as palmitoyl glucuronide, D-glucuronic acid 1-phosphate, 3-indole carboxylic acid glucuronide and 3-Methyldioxyindole. Glucuronidation, catalyzed by UDP-glucuronosyltransferase (UGT) enzymes, is a major detoxification process that liver uses to remove unwanted substances from the body [109]. The decrease of glucuronidation products in rat urine after Caowu administration indicated the disturbance of normal hepatic function [108]. The toxicity of *Aconitum carmichaelii* Debx (Fu Zi in Chinese Traditional Medicine) has to be carefully monitored. Based on the metabolomic findings, a safe therapeutic range in the clinical use of Fu Zi was established using the serum betaine and phosphorylcholine as the biomarkers [110].

4.2. *In vitro* Toxicity Screening Models

In vitro drug toxicity screening using cells is high-throughput and inexpensive [111]. Traditionally, in vitro toxicity screenings heavily relied on the estimation of cell viability in response to the test drugs. Metabolomic profiles of cell metabolism can not only be used to discriminate between non-toxic and cytotoxic drugs, but also provide the potential molecular mechanisms of drug toxicity. A metabolomics cell-based approach was developed to evaluate drug-induced liver injury using human-derived hepatic cells like HepG2 [112]. The altered concentration of glutathione and gamma-glutamyl cycle were found to be associated with oxidative stress damage caused by hepatotoxic compounds. The changes of metabolites in fatty acids β-oxidation were related to drug-induced liver steatosis. To increase the throughput, a “metabolomics-on-a-chip”

Table 2. Representative studies on drug toxicity using metabolomics.

Drug Candidates	Target Organ Toxicity	Metabolomics Platforms	Biological Fluids	Biomarkers Increased (PubChem No.)	Biomarkers Decreased (PubChem No.)	References
Caowu	Liver and heart	UPLC-Q-TOF	Urine	151066; 439211; 68759; 440443	440650; 53481645; 161223; 5282997	[108]
Phenobarbital	Liver	NMR	Urine	54670067; 152304	N/A*	[102]
Cerivastatin	Skeletal muscle	NMR	Urine	64969; 92105	N/A	[107]
N/A	Kidney	NMR	Urine	305	N/A	[103]
Phenytoin	Liver	LC-MS/MS	Plasma	N/A	N/A	[99]
Doxorubicin	Whole body	UPLC-Q-TOF	Urine	1175; 6305; 221493	464; 76970196	[105]
Griseofulvin	Liver	UPLC-Q-TOF	Liver	4971; 124886	N/A	[100]
Acetaminophen	Liver	LC-MS/MS	Serum	3035026; 2733768	N/A	[101]
Tacrolimus	Kidney	LC-MS/MS	Urine	5793; 5780 ; 1145	N/A	[106]
Fu Zi	Heart, Liver, and Kidney	UPLC-Q-TOF	Serum	247	1014	[110]

*N/A: Not applicable

approach was developed, where the cells were cultured on biochips and metabolic profiles in response to drug exposure were obtained by NMR [113].

Even though metabolomics has been used for drug toxicity evaluation for nearly 20 years, few reports were published on its application on antidepressants. This is partial because the whole society has not paid enough attention to depression and the pharmaceutical approaches for the treatment of depression are not really changed in the last 20 years. However, it is difficult to assess how many pharmaceutical companies are using metabolomics for the toxicity evaluation of potential antidepressant drug candidates or lead compounds since many cases might not be published due to the intellectual property concerns. Here, we provided a case study using albiflorin to demonstrate the feasibility of NGMin combination with traditional approaches in the toxicity assessments of antidepressant drug candidates during the early stages of drug development.

4.3. Case Study 2: Urinary Metabolomic Analysis in Combination with Conventional Approaches Revealed no Remarkable Toxicity of Albiflorin

No severe side effects were reported for Peony in thousands of years' TCM clinical practice. Albiflorin is the bioactive molecule in the roots of white peony. Studies conducted by Beijing Wonner Biotech demonstrated the antidepressant-like activities of albiflorin, which might be developed as a new class of antidepressant [15]. The potential toxicity of albiflorin was first evaluated using the conventional approaches including in vivo acute oral toxicity (Rats and dogs), in vitro mammalian cells chromosome aberration test and bone marrow cell micronucleus test (Fig. **6A**). The albiflorin dose for the conventional toxicity evaluation was 5000 mg/kg, which was 3000-fold higher than the dose for human clinical use. The results showed that albiflorin administration did not cause the significant change of Beagle dog food intake (Fig. **6B**). The blood markers such as blood urea, creatinine, lipids panel, Na^+, K^+, and Cl^- were within normal range in the albiflorin treated group. No toxic effects were observed in dog heart and kidney. Similarly, there was no significant difference in the body weight of SD rats between albiflorin treated group and controls (Fig. **6C**). The chromosome aberration test showed that albiflorin overdose did not induce any structural chromosomal abnormalities such as breaks and exchanges. The micronucleus test is the widely used in vivo genotoxicity test [114]. Albiflorin did not show any genotoxicity as there was no significant increase of micronucleated reticulocytes and micronucleated polychromatic erythrocytes.

Even though the conventional techniques did not identify any obvious toxicity caused by albiflorin overdose, the subtle detrimental toxicity can not be excluded. More sensitive NGM was then used to explore the potential toxicity of albiflorin. SD rats were administrated with 7 mg/kg of albiflorin and the urine samples were collected 12 h pre-administration and 12 h post-administration. Urine metabolites were extracted using extraction buffer containing methanol-acetonitrile-water (40:40:20, v/v/v). Briefly, 100 µl of urine was mixed with 400 µl of extraction buffer. The mixture was then incubated on ice for 10 min and then centrifuged for 10 min at 16000 g. The supernatant was then taken for further metabolomic analysis. LC-MS/MS-based metabolomics analysis was conducted on the urine samples as described before [88]. It was shown that urine metabolomic profile in post albiflorin treatment group was significantly distinct from that of the pre-administration (Fig. **7A**). Enrichment analysis was conducted using the top 30 most differential metabolites between the two groups and the search was against disease-associated metabolites database in metaboanalyst (www.metaboanalyst.ca). A total of 384 different diseases covering all the main organs were included in the search. No statistical significant hits were obtained, which suggested that albiflorin did not produce any toxic effects on rat metabolism (Fig. **7B**).

5. FUTURE DIRECTIONS

Clearly, the literature summary and cases provided in this paper have demonstrated the tremendous potential of metabolomics for drug discovery and development applications. However, as the newest addition to the "omics", metabolomics has not been widely used for new antidepressants development by the pharmaceutical industry. Indeed, only one new drug (Brexpiprazole) was approved for the treatment of depression by US FDA in the last 10 years (2008-2018) (https://www.centerwatch.com/drug-information/fda-approved-drugs/year/2018). More efforts are urgently needed to

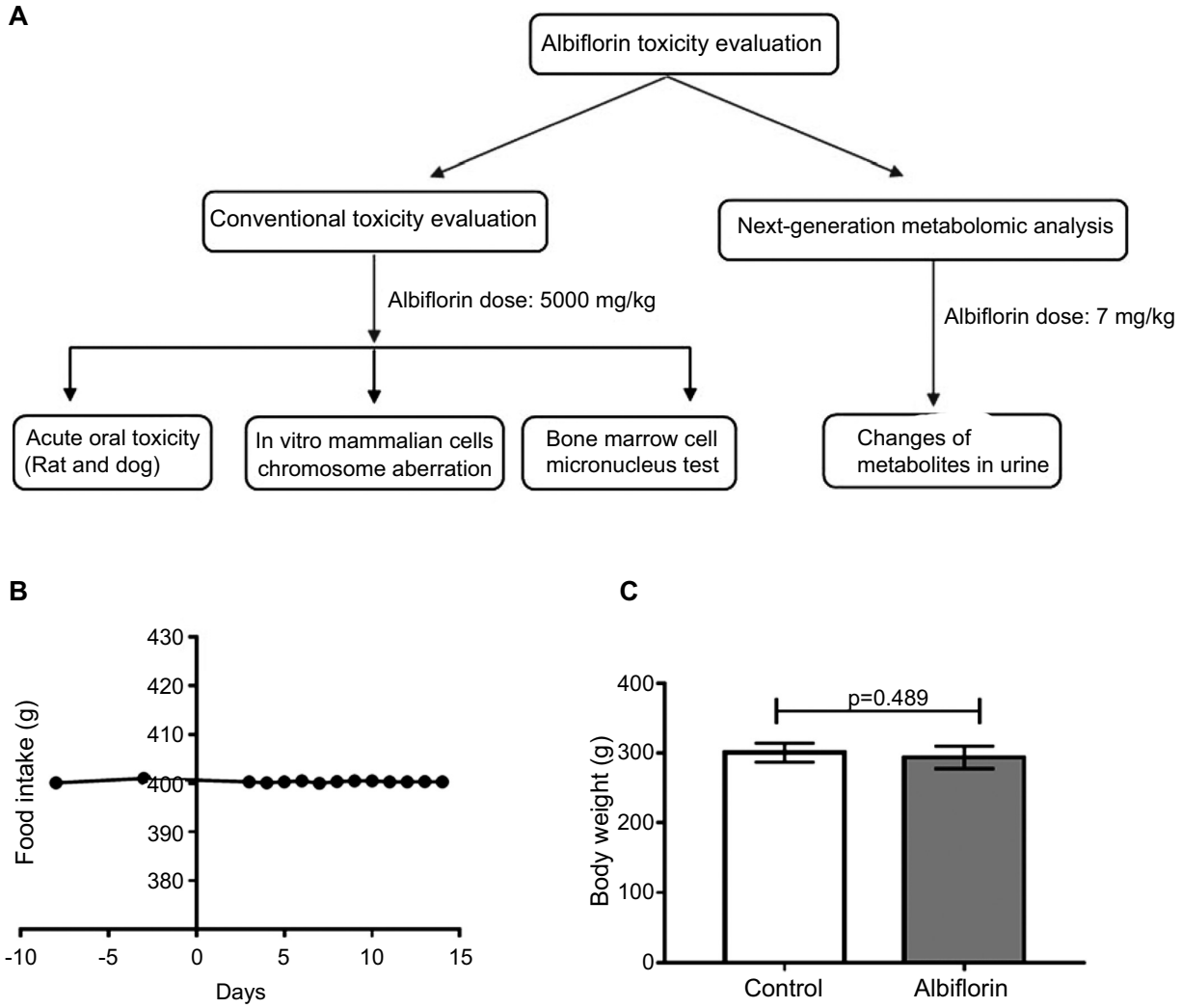


Fig. (6). The conventional *in vivo* albiflorin toxicity evaluation. **A**: The schematic diagram of the convention toxicity tests and next-generation metabolomics based test. **B**: Acute oral toxicity test showed that the food intake of Beagle dogs was not significantly changed after albiflorin administration (5000 mg/kg). **C**: Acute oral toxicity indicated that there was no significant difference on the body weight of Sprague Dawley rats between albiflorin treated group (5000 mg/kg) and controls. Student's t test was conducted. N = 5 rats per group.

enhance the productivity and creativity of antidepressants discovery and development (R&D) by metabolomics.

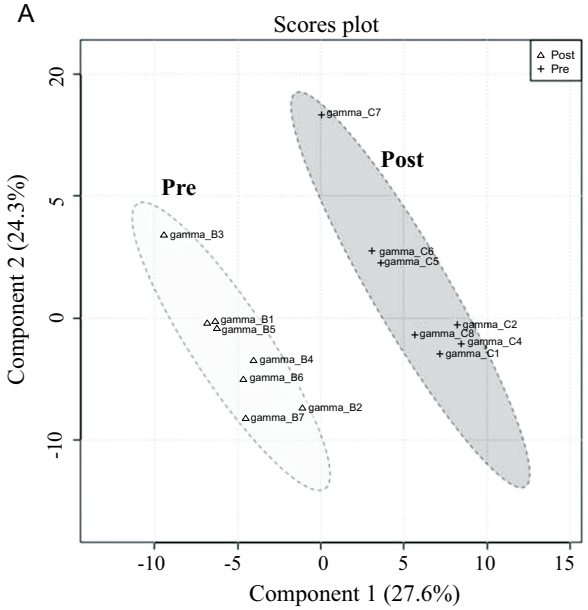
5.1. Looking Beyond the Monoamine Hypothesis of Depression

The monoamine hypothesis has dominated antidepressants R&D in the pharmaceutical companies for over 20 years, leading to the development of current first-line drugs for the treatment of depression such as SSRIs, TCAs and MOAs [115]. Recent studies revealed that the pathogenesis of depression is far beyond the monoamine deficiency. Depression is a multi-factorial complex disorder with multiple symptoms and disturbances in the brain and other parts of the body including gut microbiome [116]. In addition, depression is often accompanied by other diseases such as osteoporosis, diabetes, cancers and cardiovascular diseases [117]. Compared to the highly selective neurotransmitter-based antidepressant drugs, multi-target strategies have been proposed to be more effective control of the core and concurrent symptoms of depression [118, 119]. Botanical medicine like albiflorin has anti-inflammatory [120], anti-obesitic [121], anti-PTSD [122] and analgesic effects [123]. The multitarget nature of albiflorin makes it a strong candidate for the treatment of patients with co-existing diseases or resistant to classical antidepressant drugs.

5.2. Standardization of Metabolomic Procedures

Standardization is essential for broad application of metabolomics in drug R&D, so that the results generated by one lab and on one instrument can be validated by different labs and on different instruments. As we described in Table **2**, NGM may be conducted on different platforms using the instruments from different vendors. Moreover, the workstations and data formats are also different between vendors. Therefore, harmonization of metabolomic procedures is extremely changeling. The Wishart group from the University of Alberta provided some recommendations on NMR-based metabolomic studies using urine samples including urine collection, sample preparation and data acquisition [124]. Recently, a white paper published by "Precision Medicine and Pharmacometabolomics Task Group" on Metabolomics journal offered recommendations on the selection of metabolomic platforms, samples collection, preservation and standardization of measurements for precision medicine [125]. Standardization is still needed for each metabolomic platform, each biological fluid, data analysis, and deposition.

A



B

Diseases	Total metabolites in the pathway	Hits	Raw p	FDR
Vitiligo	9	2	0.061	1
Iminoglycinuria	3	1	0.133	1
Isovaleric acidemia	3	1	0.133	1
Xanthinuria type 1	3	1	0.133	1
Adenylosuccinase deficiency	3	1	0.133	1
Hydroxyprolinemia	3	1	0.133	1
Xanthinuria	3	1	0.133	1
Lesch-Nyhan Syndronme	4	1	0.174	1
Congenital adrenal hyperplasia	4	1	0.174	1
Pyruvate carboxylase deficiency	4	1	0.174	1
Schizophrenia	32	3	0.179	1
Dopamine hydroxylase deficiency	5	1	0.213	1

Fig. (7). The evaluation of the potential toxicity of albiflorin using next-generation metabolomics. A: PLS-DA showed the separation of urine metabolic profile between pre and post albiflorin treatment group. **B**: Enrichment analysis did not yield any significant hits indicating no potential albiflorin induced toxicity. The enrichment analysis was performed using Metaboanalyst 3.0 (www. metaboanalyst.ca).

5.3. Understanding the Biology Behind Metabolomic Big Data

Like other omic techniques, metabolomics usually generates a large set of raw data. How to obtain meaningful biological information behind the data is the key. Nowadays, for a typical metabolomic study, more than half of the total project time is devoted to the data processing and bioinformatics analysis. A comprehensive list of free metabolomic analysis tools and their usage have been summarized in several recent reviews [126, 127]. However, metabolomics is a young discipline. The tools for the functional interpretation of metabolomic experiments are still at least 10 years behind genomics, transcriptomics, and proteomics. This bottleneck limits the use of metabolomics in the pharmaceutical industry [128]. Moreover, many current metabolomic tools are run under R, Python or Java and require the users to have computer programing skills. More user-friendly tools are needed, which could provide the ability to walk through the whole data analysis process from data normalization, statistical analysis to biological pathways. In addition, in the context of omics era, tools for multi-scale and multi-omics integration will be very useful to link the phenotypes with genome, transcriptome, proteome, metabolome and gut microbiome.

CONCLUSION

Globally, investment in new drug research and discovery (R&D) falls sharply, especially for new antidepressants due to the dramatical increase of R&D expenditure and the decline of the world's economy [20, 129]. Metabolomics is entering into a "new generation" towards more sensitive, broader coverage, cheaper and more quantitative methods. NGM might be the "deal breaker" for pharmaceutical R&D.

In this review, we discussed the next-generation metabolomics from its basic concept, set up its application in antidepressant discovery and development. We also provided 2 cases to demonstrate its utility. Perhaps, with the efforts from academics, healthcare providers, pharmaceutical industries, and governments, NGM will eventually become an indispensable tool in the discovery and development of new antidepressants and really facilitate this time-consuming and pricy cycle.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

Z.Z is holding US, Europe and China patents for the use of albiflorin in the treatment of depression (China patent no. ZL2009102654220, ZL201180051467.3, ZL201080069710.X; US patent no. US9023817B2, US9453041B2, US20130231469A1, US20130316966 A1, US9555055B2 and Europe patent no. EP2491934B1). Other authors declare no competing financial interests.

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