Discovery of systematic responses and potential biomarkers induced by ochratoxin A using metabolomics

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Ochratoxin A (OTA) is known to be nephrotoxic and hepatotoxic in rodents when exposed orally. To understand the systematic responses to OTA exposure, GC-MS- and \textsuperscript{1}H-NMR-based metabolomic techniques together with histopathological assessments were applied to analyse the urine and plasma of OTA-exposed rats. It was found that OTA exposure caused significant elevation of amino acids (alanine, glycine, leucine etc.), pentose (ribose, glucitol, xylitol etc.) and nucleic acid metabolites (pseudouridine, adenosine, uridine). Moreover, myo-inositol, trimethylamine-oxide (TMAO), pseudouridine and leucine were identified as potential biomarkers for OTA toxicity. The primary pathways included the pentose phosphate pathway (PPP), the Krebs cycle (TCA), the creatine pathway and gluconeogenesis. The activated PPP was attributed to the high requirements for nicotinamide adenine dinucleotide phosphate (NADPH), which is involved in OTA metabolism through cytochrome P450. The elevated gluconeogenesis and TCA suggest that energy metabolism was involved. The up-regulated synthesis of creatinine reveals the elevated catabolism of proteins. These findings provide an overview of systematic responses to OTA exposure and metabolomic insight into the toxicological mechanism of OTA.

**Keywords:** ochratoxin A; systematic responses; biomarker; metabolic network; rats

**Introduction**

Ochratoxin A (OTA), a mycotoxin produced by several species of \textit{Aspergillus} and \textit{Penicillium}, consists of a \textit{para}-chlorophenolic moiety with a dihydroiso-coumarin group that is amide-linked to L-phenylalanine (Ringot et al. 2006). As a natural food contaminant, OTA exists widely in a variety of food items, predominantly including cereal grains and products, spices, coffee beans and legumes (Duarte et al. 2010b). According to reports of the European Union (Duarte et al. 2010a), humans are chronically exposed to OTA in the dose of 1 ng kg\(^{-1}\) b. w. for the average adult and 6–8 ng kg\(^{-1}\) b.w. for certain consumers. Numerous studies have shown that OTA exerts a diverse range of toxicological effects including nephrotoxicity (Baudrimont et al. 1994), hepatotoxicity (Shlosberg et al. 1997), carcinogenicity (Bendele et al. 1985), teratogenicity (Mayura et al. 1984), mutagenicity (de Groene et al. 1996), and immunotoxicity (Lea et al. 1989). In rodents, OTA is considered to be both a nephrotoxin and a potent renal carcinogen. A 2-year carcinogenesis study demonstrated that there is a clear causative relationship between OTA exposure and the development of renal pathologies (Boorman et al. 1992). However, the carcinogenic mechanism of OTA remains elusive. The OTA-induced carcinogenesis are thought to be a direct (covalent DNA adduction) or indirect (oxidative DNA damage) effect (Turesky 2005). Due to the failure to demonstrate the formation of OTA-derived DNA--adducts \textit{in vivo}, it is widely and preferably acknowledged that the oxidative damage plays a central role in OTA-mediated carcinogenesis (Mally 2012). Cytochrome P450 (CYP450) enzymes were considered to be primarily responsible for the oxidation of OTA (Omar et al. 1996). The OTA metabolites such as 4(R)-, 4(S)- and 10-OH-OTA, ochratoxin hydroquinone (OTHQ) and OTA derived from different biotransformation of CYP450 isoforms in different tissues or cells. Then these metabolites can induce the formation of reactive oxygen species (ROS) or covalent DNA adduction. Furthermore, some studies (Baudrimont et al. 1994; Palma et al. 2007) have demonstrated the induction of oxidative stress by OTA. Therefore, the mechanism of OTA nephrotoxic and carcinogenic toxicity is probable caused by the oxidative stress induced by OTA.

Metabolic profiling is a useful tool to study toxicity as it provides a unique mechanistic perspective on responses to toxic insult. In recent years, metabolomics

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has been widely applied to uncover biomarkers (Boudonck et al. 2009) and metabolic fingerprint in drug discovery and clinical toxicology (Beger et al. 2010), especially to investigating systematic metabolic responses to toxins (Zhang et al. 2010) and the associated mechanisms (Huang et al. 2013). A metabolomic investigation conducted by Sieber et al. (2009) demonstrated that OTA (210 µg kg⁻¹ b.w.) can cause the metabolic changes after 14 days of exposure to OTA at 210 µg kg⁻¹ b.w. These metabolic changes include two intermediates in the TCA and several potential biomarkers. A metabolomic study on acute toxicity shows a single 6.25 mg dose of OTA by oral gavage induced the fluctuation of creatinine and trimethylamine N-oxide’s levels in urine (Mantle et al. 2011). However, the global differences in metabolic pathways by OTA are still not clear. A series of studies were conducted to explore the systematic responses to OTA exposure from proteomic, transcriptional and metabolic perspectives. The analysis on differentially expressed miRNAs in kidney indicated that the mitogen-activated protein kinase (MAPK) signalling pathway may play a vital role in mediating OTA nephrotoxicity (Dai et al. 2014). The sequencing data suggest that five distinctive pathways (primary bile acid biosynthesis, metabolism of xenobiotics by cytochrome P450, arginine and proline metabolism, cysteine and methionine metabolism, and the PPAR signalling pathway) were induced after OTA treatment as collectively demonstrated at mRNA, mRNA and protein levels, which contributed to OTA-induced early hepatotoxicity (Qi et al. 2014). In addition, the metagenomics study on gut microbiota showed that OTA can cause compositional and functional changes of gut microbiota, and Lactobacillus are key genus to detoxify OTA in vivo (Guo et al. 2014).

The primary goal of this study is to study systematically the metabolic pathway changes induced by OTA in male F344/N rats after 2, 4, 13 and 26 weeks of treatment. Here, a metabolomic approach coupled with histopathological tests was used to screen and identify metabolic perturbations associated with OTA-induced toxicity in rat biofluids. The results from histophysiological analyses showed no distinguishable pathological changes, but the patterns of the metabolites in urine and plasma were altered after 2 weeks of OTA administration. Several metabolic pathways involved in the nephrotoxicity of OTA were altered.

Materials and methods

Animals and sample collection

Male F344/N rats (6–7 weeks old, vitalriver, Beijing, China) were housed in an SPF animal room at 22 ± 2°C with a RH of 40–70% under a cycle of 12 h light/dark and air exchanges of 15 times/h. Food and tap water were provided ad libitum. Body weight was recorded once a week. After acclimatisation for 1 week, the rats (six per group) were administered with OTA at doses of 0, 70 or 210 µg kg⁻¹ b.w. in corn oil (Aladin, China) by gavage for 2, 4, 13 or 26 weeks (5 days per week). To make it simple, we used A, B, C and D to name the groups of 2 weeks, 4 weeks, 13 weeks and 26 weeks respectively. C (control group), L (low-dose group) and H (high-dose group) represent the groups of 0, 70 and 210 µg kg⁻¹, respectively. All experimental procedures involving animals were approved by the Ethics Committee of China Agricultural University. The permission number of the animal study is 120020.

Samples collection and treatment

Urine samples were collected during a 24-h collecting period after 2, 4, 13 or 26 weeks of administration with the addition of 20 µl sodium azide solution (0.5% w/v) in individual metabolic cages (one animal per cage). During urine collection, animals were fasted but had free access to drinking water. At 2, 4 and 13 weeks after OTA treatment, blood samples were collected from the orbital plexus into Eppendorf tubes with and without sodium heparin to obtain plasma and serum samples, respectively, with standard centrifugation procedures (1660g, 10 min, 4°C). The tissue sections of liver and kidney were fixed in 4% buffered formaldehyde, embedded in paraffin and stained with hematoxylin and eosin. The urine and plasma samples were stored at −80°C for later analysis.

GC-MS analysis

The urine samples were thawed at 4°C, followed by a vortex to mix each urine sample. Aliquot 200 µl of each urine sample was transferred into individual 2 ml microcentrifuge tubes. Urine samples were incubated with 20 µl of urease suspension (equivalent to 100 U of urease enzyme) for 1 h, then urease and other proteins were precipitated with 1.7 ml of ethanol and the supernatant is dried under nitrogen and redissolved in 50 µl of pyridine containing 20 mg ml⁻¹ methoxyamine hydrochloride. Samples were derivatised at 40°C for 90 min. The samples were then silylated at 40°C for 1 h with 100 µl of N-Methyl-N-(trimethylsilyl)trifluoroacetamide and used directly for GC-MS analysis. Additionally, quality control (QC) samples was prepared by pooling 50 µl of all the urine samples, which were treated the same as the urine samples as described before. QC samples were randomly applied into each sequence (eight samples) to monitor the performance of data acquisition.
After derivatisation, the extracts were analysed using an Agilent 6890 gas chromatograph equipped with an HP5973 mass selective detector. The column used was a DB5-MS (30 m × 0.25 mm × 0.1 µm). The carrier gas was He with the flow rate of 1.0 ml min⁻¹. Solvent delay was set for 4.5 min. The injection port was at 280°C. The oven temperature was programmed as follows: initially 40°C, increased to 80°C at 2.0°C min⁻¹, and held for 2 min; increased to 210°C at 2.5°C min⁻¹, and held for 2 min, then increased to 280°C at 4°C min⁻¹. The mass (MS) detector was used for qualitative analysis. Data acquisition was performed in full-scan mode from m/z 50 to 550. The ion source and quadrupole temperatures are 230 and 150°C, respectively.

After deconvolution by the automatic mass spectral deconvolution and identification system, the raw data of GC-MS were imported into Mass Profiler Professional software (MPP, Agilent) for further analysis. Retention time alignment, baseline correction, noise reduction and peak alignment and area calculation were performed by MPP. The molecular features that existed in at least 75% of the samples in either group were retained. Subsequently, principal component analysis (PCA) was applied to examine any intrinsic clustering of the data. Furthermore, partial least squares-discriminant analysis (PLS-DA) was applied in order to visualise the maximal difference between OTA exposed and control groups. For potential biomarker discovery, the selected metabolites were filtered based on fold change (FC) > 2 and p < 0.05.

**Results**

**Histopathological test**

In rats treated with OTA for 13 (Dai et al. 2014) and 26 weeks (Figure 1), the kidney showed cytoplasmic vacuolisation in the outer stripe of outer medulla (OSOM) and karyomegaly in the tubular epithelium. Moreover, this damage appears to be time- and dose-dependent. As shown in Figure 1, 26 weeks after treatment with OTA at a concentration of 210 μg kg⁻¹ b.w., the karyopyknosis was also noted in the tubular epithelium. However, on the second and fourth weeks, there were no visible differences in histopathology between the control (AC and BC) and the administrated (AL, AH, BL and BH) groups. In contrast, all rats showed no distinguishable pathological changes in the liver (data not shown).

**1H-NMR analysis of rat plasma metabolome**

The different metabolism patterns between the control and OTA-exposed groups can be recognised through multivariate statistical analysis. On the second, fourth and 13th weeks, the control groups and OTA-exposed groups appeared to be overlapped with each other in the score plots of PCA, which indicated that no obvious profile separations were found between the control and OTA-exposed groups at all three time points. On the contrary, when ¹H-NMR spectral data were further...
analysed by orthogonal partial least squares discriminant analysis (OPLS-DA), the obtained plots showed a better separation for all the investigated groups except that the OPLS-DA of CPMG $^1$H-NMR spectral data showed partly overlapped with each other in the score plot at the fourth week. In addition, $Q^2$ of the model was only 26.2% (Figure 2e), suggesting that the model did not have high predictive capacity. For differentiating the metabolic profiles of different groups, the OPLS model worked more efficiently than the PCA model. Representative score plots of PCA and OPLS-DA are both shown in Figure 2. Together with the corresponding loading plots, OPLS analysis indicated metabolic differences among the control, low- and high-dose groups on the second, fourth and 13th weeks. As listed in Table 1, the metabolic differences

![Figure 2](image_url)
between the control and OTA-exposed groups are mainly glycoproteins, glucose, amino acids and carboxylic acids, such as lactate, iso-butyrate, alanine, TMAO, threonine and creatinine. These discriminating metabolites might be used for separation between the control and OTA-exposed groups and for metabolic evaluation of OTA toxicity. Nevertheless, these metabolites did not reveal a significant time- and dose-dependent response to OTA. Some of the metabolites even showed the opposite trends at different time points. For example, the concentration of lactate increased in OTA-exposed groups after 2 weeks and then decreased after 13 weeks. This condition might be attributed to the OTA-induced perturbation of different pathways at different time points.

**GC-MS analysis of rat urinary metabolome**

To explore the changes in endogenous metabolite levels caused by OTA, PCA was carried out. The PCA score plots of urine samples are shown in Figure 3. Compared with the control groups, OTA-exposed groups were located separately as early as 4 weeks. On the contrary, the histophysiological test showed differences after 13 weeks of treatment at a concentration of 210 μg kg−1 b.w., which indicated metabolomics was more sensitive to revealing the toxic effects of OTA. According to PCA analysis, the metabolic differences in urine between the OTA-exposed and control groups had occurred since 4 weeks of OTA treatment. The significantly changed (FC > 2 and p < 0.05) metabolites were screened and are displayed in Table S2 in the online Supplementary Data. Compared with the control groups, the primary differences in OTA-exposed groups were the increase of D-glucose, myo-inositol, and the decrease of hexanedioic acid and pentanedioic acid.

To investigate the dose and time–response relationship that OTA impacts on metabolic changes, the groups administered with the same dose among different time points were compared using the PCA model. In the PCA model, the low- and high-dose groups also showed a separation trend during all time points (Figure 4), which indicated that the metabolic changes perturbed by OTA have time-dependent responses at both doses. According to the analysis above, the crucial metabolites responsible for these profile separations from urine metabolome were identified. The compounds (the p-value of the t-test was less than 0.05) were selected as discriminating compounds, as summarised in Tables 2 and S3 (the latter in the online Supplementary Data).

**Table 1. Differential metabolites in plasma between control and OTA-exposed groups at 2, 4 and 13 weeks based 1H-NMR.**

| Chemical shift | Compound | AH versus AC | AL versus AC | BH versus BC | BL versus BC | CH versus CC | CL versus CC |
|----------------|----------|---------------|--------------|--------------|--------------|--------------|--------------|
| 1.31–1.33, 4.09–4.13 | Lactate | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ |
| 1.16–1.18 | Iso-butyrate | ↓↓↓↓ | ↓↓↓↓ | ↓↓↓↓ | ↓↓↓↓ | ↓↓↓↓ | ↓↓↓↓ |
| 1.47 | Alanine | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ |
| 2.22 | Acetone | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ |
| 3.26 | TMAO | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ |
| 1.26–1.3 | Threonine | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ |
| 0.86–0.88 | Lipid | –––– | –––– | –––– | –––– | –––– | –––– |
| 3.22 | PC/glucose | ↓↓↓↓ | ↓↓↓↓ | ↓↓↓↓ | ↓↓↓↓ | ↓↓↓↓ | ↓↓↓↓ |
| 0.03 | Creatinine | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ |
| 3.18, 3.22, 3.66 | Phosphatidylcholine | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ |
| 2.06 | N-acetyl-glycoprotein | ↓↓↓↓ | ↓↓↓↓ | ↓↓↓↓ | ↓↓↓↓ | ↓↓↓↓ | ↓↓↓↓ |
| 2.22, 2.26 | Saturated fatty acid | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ |
| 2.14, 2.18 | O-acetyl-glycoprotein | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ |
| 2.13 | Oac | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ |
| 2.02 | Unsaturated fatty acid | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ | ↑↑↑↑ |

Note: AC, control groups at 2 weeks; AL, low-dose group at 2 weeks; AH, high-dose groups at 2 weeks; BC, control groups at 4 weeks; BL, low-dose groups at 4 weeks; BH, high-dose groups at 4 weeks; CC, control groups at 13 weeks; CL, low-dose groups at 13 weeks; and CH, high-dose groups at 13 weeks.

**Pathways analysis**

Comparing metabolic profiles using 1H-NMR and GC-MS analysis, it was assessed whether the major differences exist in response to biological systems caused by OTA. Based on these differential metabolites, a correlation network diagram was constructed. Several metabolic pathways in response to OTA are presented in Figure 5. An increase in pentose phosphate pathway (PPP) metabolites, including sedoheptulose, gulonolactone, ribitol, ribose and xylitol, was observed. However, glucose, xylose and gulonic acid, which were also
involved in the PPP, decreased simultaneously. Furthermore, the levels of glucogenic amino acids, such as alanine, glycine, serine, threonine and aspartate, which are related with gluconeogenesis, were significantly increased in the OTA-exposed groups. In addition, the amino acids involved in the synthesis of creatine were increased, consistent with the elevated level of creatinine.
Discussion

It is widely recognised that OTA is a potent nephrotoxic mycotoxin that induces renal tumours in rodents. Omics technologies have been applied in several studies on the toxicity of OTA that have addressed the OTA-mediated fluctuations in the expression of proteins (Shen et al. 2012) and mRNAs (Jennings et al. 2012). However, it is still not clear about the OTA-induced systemic changes of the metabolites in rats. In the absence of adequate metabolomics data, time and dose effects of OTA become hard to evaluate. The current study aims to investigate systematically the metabolic differences mediated by OTA. We have examined metabolic alterations in extensive biological matrices after OTA treatment including plasma at the second, fourth and 13th weeks and urine samples at the second, fourth, 13th and 26th weeks. GC-MS and

\[ ^{1}H\text{-NMR} \]

were conducted on urine and plasma samples with the aim to discover the metabolic changes of OTA-induced nephrotoxicity and globally altered metabolism, respectively.

As shown in Table S1 in the online Supplementary Data, the data of serum biochemistry analysis dose not display any time or dose-dependent alterations clearly. Blood urea nitrogen and creatinine (BUN), common indexes of kidney damage, do not reflect the nephrotoxicity induced by OTA. Nevertheless, on the basis of the histophysiological result, OTA is toxic to the kidney, which caused renal injuries after 13 weeks of gavage at least at the dose of 70 μg kg\(^{-1}\) b.w. (Dai et al. 2014). In contrast, the hepatic injury cannot be detected in OTA-exposed groups. In one word, the pathological examination revealed that OTA can cause kidney damage after
13 weeks of treatment. At the same time, the histopathological test on the liver showed a negative result, suggesting that the kidney is more sensitive to OTA than the liver. This is consistent with the previous reports by the NTP (1989) and Rached et al. (2007). In the NTP study, 0, 21, 70 and 210 μg kg⁻¹ b.w. OTA were gavaged to rats for 2 years. At the 15th month, male rats treated with 70 and 210 μg kg⁻¹ b.w. OTA were found with renal tubular cell adenomas and carcinomas of the renal tubular epithelium. On days 28 and 90, minimal to moderate histopathology changes were found in the kidney, which was consistent with the present study. Similarly, Rached et al. (2007) found renal alterations in the same dose-treated animals involving single-cell death and prominent nuclear enlargement within the straight proximal tubules. Both of these results can support the abovementioned viewpoint. In addition, severe damage of the kidney structure in male rats occurred in OTA-exposed groups in this study. However, nephrotoxic and hepatotoxic indexes of serum clinical chemistry were not significantly changed. Even so, the multivariate data analysis of metabolic profiling on plasma and urine demonstrates the metabolic differences, as is shown in Figures 2 and 3. Furthermore, detection of the biomarkers by metabolomics could be prior to the appearance of lesions or damage revealed by histological assessments and traditional clinical biochemical assays.

According to Figure 5, several amino acids and carbohydrates were elevated by OTA treatment. OTA is known to reduce glucose and amino acid reabsorption in the kidney due to its toxic effect on the proximal tubule. However, the appearance of metabolites in the urine is not simply a presentation of increased glomerular filtration, but rather represents a complicated equation of utilisation and production of the compound. The main metabolic pathways OTA altered are PPP, TCA, the creatinine pathway and gluconeogenesis. As Figure 5 presents, the majority of metabolites in PPP were significantly enriched, suggesting the more metabolically active PPP to provide NADPH. It is well acknowledged that oxidative stress induced by OTA plays an important role in nephrotoxicity (Schaaf et al. 2002). In consideration of the key role of the cytochrome P450 (CYP450) in OTA metabolism, the OTA biotransformation of CYP450 would be observed in the OTA-exposed groups. The metabolism of OTA by CYP450 then needs NADPH as the hydrogen donor, which is mainly produced through PPP. Therefore, it can be speculated that PPP is up-regulated in response to the strong demand for the detoxication of OTA. In addition, the amount and isoforms of CYP450 are origin and microsome-specific. The biotransformation of OTA mostly depends on the isoforms of CYP450, and the OTA derivatives can be genotoxic and mutagenic through the formation of DNA adducts or non-toxic conjugation to glutathione and/or uridine diphosphate (Pfohl-Leszkowicz & Manderville 2007). The origin involved in the OTA biotransformation by CYP450 is principally the liver, which can be responsible for the histopathological test, except for the kidney-specific enrichment. The sequencing data of multi-omics suggested that some of the proteins, miRNA and mRNA involved in the metabolism of xenobiotics by CYP450 were perturbed in response to OTA, which provide a pathophysiological explanation to OTA-induced live damage (Qi et al. 2014). Meanwhile, glucose in both plasma and urine is decreased compared with the control groups. A plausible explanation is that the up-regulation of PPP required more glucose to consume, resulting in down-regulation of energy metabolism concerning glucose utilisation. In addition, the increased levels of glucogenic amino acids also indicated the insufficiency of glucose in energy metabolism. However, the key metabolites in glycolysis such as glucose-6-phosphate and pyruvate did not change significantly, and many other metabolites in TCA were up-regulated. The gluconeogenesis attributable to the glucogenic amino acids effectively replenishes the shortage of glucose when consumed by metabolically active PPP. These results illustrate that the level of energy metabolism is elevated. Another interesting phenomenon in OTA-exposed groups is the up-regulation of creatinine synthesis. The level of creatinine in plasma was not found to be significantly changed; however, it showed a slight dose-dependent increase in the fourth week. In contrast, a statistically significant increase with a cumulative effect as time proceeded was detected in the urine of low-dose groups. Stoev et al. (1998) observed increases in urea and creatinine in the blood and urinary excretion in pigs with impairment of proximal tubular function. In contrast, the levels of serum protein and glucose were decreased in the pigs. Creatinine, an end product of protein metabolism, rests with the catabolism of nitrogen and the excretory capability of kidney (Jia et al. 2008). The relatively stable concentration of creatinine in plasma suggests that the excretory capability of kidney has not yet functionally damaged in the 13th week. However, the raised concentration in urine reveals that the catabolism of protein was up-regulated, which is consistent with the elevated levels of the glucogenic amino acids. Besides that, our transcriptomics study on liver suggested that OTA influences energy metabolism mainly through the pathway of carbohydrate metabolism, and basic substance metabolism (including amino acid metabolism, metabolism of cofactors and vitamins) may be another potential mechanism by which OTA-induced liver damage (Qi et al. 2014). Based on the same animal experiments, microRNA profiling showed that the MAPK signalling pathway may play a vital role in mediating OTA toxicity in kidney (Dai et al. 2014). Meanwhile, the metagenomics sequencing showed that OTA can cause compositional and functional changes of gut microbiota, and Lactobacillus are key genus to detoxify OTA in vivo (Guo et al. 2014). Taken together, the toxic mechanism...
of OTA was systemically studied through multi-omics methods; however, the differential pathways and the potential mechanisms did not appear fully consistent in different tissue or biofluids. Nevertheless, these studies exhibit an overview of systematic responses to OTA exposure from proteomic, transcriptional and metabolic perspectives and provide a better insight into toxicological mechanism of OTA.

Previous studies showed that the levels of myo-inositol and pseudouridine were significantly changed after exposure of OTA (myo-inositol: 1.5-fold increase in all treated animals after 2 weeks and two- and three-fold increases in high-dose animals at 4 and 13 weeks; pseudouridine: 1.3-fold increase at 4 and 13 weeks). Myo-inositol is one of the most abundant osmolyte excretions, which is the biomarker of early toxic effect (Waters et al. 2005). Consistent with this, myo-inositol displays a statistically significant rise in high-dose group after 13 weeks in this study and the histophysiological test showed that the structure of tubular epithelium in group of gavaging rats for 26 weeks with 210 μg kg⁻¹ b.w. OTA was severely damaged. Taken together, myo-inositol can be considered as a candidate biomarker for structural damage on kidney tubules. Pseudouridine is a modified nucleoside found in ribosomal and transfer RNA and is produced post-transcriptionally, which is an excellent measure for RNA degradation and thus of cell turnover and also be proposed as a marker of renal cell proliferation (Dunn et al. 2007). Besides these two candidate biomarkers, increased TMAO was also be detected in both of the OTA-exposed groups by ¹H-NMR metabonomics. The biosynthesis of TMAO starts from the degradation of dietary choline, which is first metabolised to trimethylamine (TMA). TMAO is an oxidation product of TMA and then excreted in the urine. The change of TMAO often occurs due to the drug-induced nephrotoxicity (Smith et al. 1994; Wang et al. 2013). However, the level of TMAO shows different trends of variation among the studies of nephrotoxicity induced by different chemicals. The increased amino acids in urine are supposed to be one of the strongest nephrotoxin-induced responses and an excellent potential marker for very early kidney malfunction and nephrotoxicity. Amino acids that showed significant increases in urine in the different drug groups include serine, threonine, alanine, asparagine, leucine, ornithine and proline. Moreover, leucine, one of the branched-chain amino acids, is a biomarker involved in the model that predicts early nephrotoxicity effects.

To sum up, although the pathological changes in kidney were observed after 13 weeks in the histophysiological test, the changes of metabolite can be detected as early as 2 weeks. Of these differential metabolites, myo-inositol, TMAO, pseudouridine and leucine are regarded as potential biomarkers of OTA. Based on the metabolic approach, the elevated PPP could be both relevant to OTA metabolism and CYP450 system induction for counteracting OTA-induced oxidative stress, while the up-regulated gluconeogenesis and creatinine synthesis reveal that protein catabolism complements the lack of glucose to meet the high demand of energy metabolism.

Conclusions

In this study, a ¹H-NMR- and GC-MS-based metabolomic approach coupled with a pathological study and serum clinical chemistry was employed to investigate the systematic metabolic responses to OTA. The histopathological test and serum clinical chemistry showed that OTA induced time- and dose-dependent damage on kidney. Myo-inositol, TMAO, pseudouridine and leucine were discovered as potential biomarkers. Exposure to OTA induces significant alterations in several metabolic pathways, including the PPP and gluconeogenesis, which demonstrated that OTA modified the glucose metabolism to meet the requirement of OTA metabolism and enhance energy metabolism level. These findings systematically demonstrate the metabolic differences mediated by OTA and provide the holistic metabolomic information for elucidating the mechanisms of OTA-induced toxicity.

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Supplemental data

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