Ochratoxin A Induces Steatosis via PPARγ-CD36 Axis

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Ochratoxin A(OTA) is considered to be one of the most important contaminants of food and feed worldwide. The liver is one of key target organs for OTA to exert its toxic effects. Due to current lifestyle and diet, nonalcoholic fatty liver disease (NAFLD) has been the most common liver disease. To examine the potential effect of OTA on hepatic lipid metabolism and NAFLD, C57BL/6 male mice received 1 mg/kg OTA by gavage daily. Compared with controls, OTA increased lipid deposition and TG accumulation in mouse livers. In vitro OTA treatment also promoted lipid droplets accumulation in primary hepatocytes and HepG2 cells. Mechanistically, OTA prevented PPARγ degradation by reducing the interaction between PPARγ and its E3 ligase SIAH2, which led to activation of PPARγ signaling pathway. Furthermore, downregulation or inhibition of CD36, a known of PPARγ, alleviated OTA-induced lipid droplets deposition and TG accumulation. Therefore, OTA induces hepatic steatosis via PPARγ-CD36 axis, suggesting that OTA has an impact on liver lipid metabolism and may contribute to the development of metabolic diseases.

Keywords: fatty liver disease ; lipid metabolism ; OTA ; PPAR

1. Introduction

Ochratoxin A (OTA) is produced by several species of Aspergillus and Penicillium [1], and is one of the most common mycotoxin contaminant in food. It has been identified in various crops, including cereals and cereal products, coffee beans, peanuts, dried fruits, spices, legumes, wine and beer [2]. OTA has long been studied as a nephrotoxin, immunotoxin, teratogen and carcinogen in humans as well as other animal species [3][4][5][6], and is regarded to be a nonnegligible risk of human health because of its widespread occurrence. This mycotoxin is metabolized and accumulated mainly in the liver and kidney [7], which are the major target organs for OTA [8].

Liver is a vital metabolic organ in the maintenance of whole-body homeostasis. Because liver is responsible for metabolism, distribution and excretion of exogenous chemicals, it is threatened by significant concentrations of chemicals, and chemical- or drug-induced liver injury (hepatotoxicity). Furthermore, it is recently suggested that nonalcoholic fatty liver disease (NAFLD), or steatosis, is the most prevalent pathology associated with toxicant exposure [9]. In particular, OTA affects hepatocytes via multiple pathways, including oxidative stress [10][11], inflammation [12], apoptosis [13][14][15] and genotoxic effect [16][17], It is reported that OTA would increase the expression of genes involved in the synthesis of fatty acid in kidney. In contrast, it significantly inhibited the expression of genes related to fatty acid oxidation [18]. However, the lipotoxicity of OTA in liver remains unknown.

Peroxisome proliferator-activated receptors (PPARs) are nuclear hormone receptors that are activated by fatty acids and their derivatives [19]. There are three PPAR isotypes—α, β/δ and γ, they are well known to serve as important regulatory factors of lipid metabolism. PPARα modulates transcription of specific target genes involved in lipid oxidation, lipid transport, lipoprotein assembly and ketogenesis [20]. PPARβ/δ is most abundant in metabolically active tissues such as skeletal and cardiac muscle, and regulates lipid metabolism, inflammation and oxidative stress responses [21][22]. PPARγ plays a role in regulating adipocyte differentiation and energy storage in mature adipocytes [23]. Thus, PPARs are promising drug targets for the management of NAFLD.

The influence of OTA on hepatic lipid metabolism was investigated. It's found that OTA increased lipid droplets deposition and TG accumulation in primary hepatocytes and HepG2 cells, and induced steatosis in mice. The mechanistic study revealed that OTA disturbed lipid metabolism in liver cells mainly through PPARγ-CD36 axis. OTA can stabilize PPARγ via preventing its ubiquitination and subsequent degradation. Therefore, our study provides novel insights into the mechanism underlying the disturbance of hepatic lipid metabolism by OTA.

2. Insights and Summary

OTA is considered to be one of the most important contaminants of global food and crops. Ambient temperature, humidity, food storage and transportation may promote fungal growth leading to increased occurrence of OTA in various crops [24]. OTA has been detected in human blood and serum in Canada, Sweden, West Germany and Yugoslavia [25], suggesting the high incidence of OTA exposure in human. Therefore, there is a need to investigate the toxic effects of OTA for prevention.

Previous studies reported that inhibition on protein synthesis and energy generation, induction of oxidative stress, apoptosis/necrosis, DNA adduct formation and cell cycle arrest were possibly involved in OTA toxicity. OTA intake increased some marker of liver damage such as AST, ALT, GGT and ALP [26], which may be caused by OTA-induced oxidative damage [27] and apoptosis [28]. OTA was reported to enhance lipid peroxidation [29][30], however, its influence on other aspects of lipid metabolism remains largely unknown. In the present study, we found that OTA increased lipid deposition and TG accumulation in liver, which revealed its influence on hepatic lipid metabolism and its risk to induce NAFLD. These findings have improved our understanding of this fungal toxin.

PPARs are representative members of nuclear receptors. This large superfamily is capable of ligand binding, which modulates their activities to regulate gene expression [31]. It has been determined that fatty acids and their derivatives bind and activate PPAR proteins [32]. Therefore, PPARs are important regulators to maintain cellular metabolic homeostasis. Lim et al. reported that OTA notably reduced the expression of adipocyte-specific genes, including PPARγ, therefore inhibited adipogenesis in mesenchymal stem cells derived from human adipose tissue [33]. In contrast, we found that PPARγ protein expression was increased in the livers after OTA treatment, whereas the mRNA level was comparable with control livers. This inconsistence may be attributed to the different cell types. It was reported that prolonged OTA exposure decreased ubiquitination levels of proteins by promoting proteasome activity [34]. However, we observed that OTA increased the PPARγ protein level in our study. We found that the interaction between PPARγ and its E3 ligase SIAH2 was reduced upon OTA treatment. Consequently, OTA prevented degradation of PPARγ. Therefore, OTA may influence protein stability in different ways.

Consistent with the increased expression and activity of PPARγ upon OTA treatment, the expression of CD36, a target of PPARγ [35] was increased in vivo and in vitro upon OTA treatment. CD36 is an important mediator of lipid uptake in many tissues, and abnormal CD36 expression in the liver resulted in TG accumulation and the development of hepatic steatosis [36]. As expected, OTA-induced lipid droplets formation and TG accumulation was alleviated in CD36 knockdown cells. FABP2 is involved in fatty acid transportation [37], and is another downstream target of PPARγ. Similar to CD36, expression of FABP2 was also increased after OTA treatment. Knockdown of *FABP2* reduced lipid droplets accumulation, but had no effect on TG contents. We noticed that OTA also upregulated the expression of two other FABPs, FABP1 and FABP3 (**Figure 4**d), although the alteration was less significant than FABP2. These FABPs may compensate for knockdown of FABP2, which contributed to the modest effect on lipid metabolism caused by FABP2 silencing. Therefore, CD36 seems the predominant effector downstream of PPARγ to mediate the effect of OTA on hepatic lipid metabolism.

In summary, the current study demonstrated that long-term exposure to OTA induces lipid accumulation in the liver of mice, mainly through activation of PPARγ signaling via post-translational modification of this nuclear receptor. The study not only reveals the novel hepatic toxicity of OTA other than ROS generation and apoptosis induction, but also highlights the risk of OTA to cause NAFLD.

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