Ochratoxin A (OTA) Affect OATPs and PEPTs and Cause Renal Disease

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Abstract

Ochratoxin A (OTA) is a mycotoxin related to the kidneys. There is a consensus that the primary route in which OTA is excreted into the urine is via tubular secretion, possibly through the organic anion transport system. There are some kinds of multipiece organic anion-transporting polypeptides (OATPs), ATP-binding cassettes (ABC) transporters, and transporters such as organic anion transporters (OATs). These renal transporters are essential in exacerbating OTA-induced nephrotoxicity as OTA penetrates the cell membrane. This work concluded that the renal tubule's reabsorption and secretion of OTA might play an essential role in OTA accumulation and nephrotoxicity. Also, it tells us it is essential to consider the impact of albumin binding on transport in vitro when considering the clearance of OTA in vivo.

Keywords: OTA, OATPs, OATs, PEPTs

1. Introduction

A food-contaminating mycotoxin, Ochratoxin A, is produced by different Aspergillus and Penicillium species[1]. Food contamination is able to introduce contaminants into the human system through the ingestion of contaminated grain, wine grapes, or dried grapes. In addition to human blood and breast milk, animals' organs and tissues, the toxin has been found. The toxicological profiles of ochratoxin A differ greatly depending on the species and sex. This toxin binds strongly to the brain, particularly to Purkinje cells in the hippocampal structures and cerebellum[1,2]. Ochratoxin A is found to be thermally/hydrolytically stable, although the instability of ochratoxin A after grinding of grains during the preparation of test samples was one of the challenges encountered in developing analytical methods for ochratoxin A in grains. Some loss of ochratoxin A appears in the processing of flours contaminated with ochratoxin A. Induces neurodegenerative diseases of the hippocampal region in rodents after chronic administration because of its affinity for the hippocampus. As a result of ochratoxin exposure, striatal dopamine is quickly depleted, which is the basis for Parkinson's disease[3]. However, no cell death was observed in any of the brain regions examined. A major component of the kidney is the proximal tubule, which is responsible for the reabsorption of most of the glomerular ultrafiltrate and the secretory transport of many substances that are essential for the fluid-electrolyte and acid-base regulation of the body. A kidney is an organ that Provides the body with chemicals and waste to remove from the blood, and removes waste and water through urine[4]. Additionally, kidneys produce hormones and red blood cell production is stimulated by these hormones, which help control blood pressure. Malignant kidney tumors are cancerous kidney tumors. Renal cell cancer and transitional cell cancer are very prevalent in renal disease. Renal cell cancer, which develops in the small tubes inside the kidney, is one of the most common diseases among the adult[5]. A rarer type is transitional cell cancer. Children are most often affected by Wilms' tumor, another type of kidney cancer[6,7].

Cancerous kidney tumors are malignant kidney tumors. Cancer of the kidneys is most commonly found as renal cell carcinoma and transitional cell, which develop in the lining of small tubes within other devices used to see and clean the tumors. It has been shown that one kind of anatomy surgery is very effective as traditional surgery and makes for an easier recovery[8].

Organic-anion-transporting polypeptides (OATPs) called Membrane transport proteins, that relieve the transportation of ions through the cell membrane are members of the Organo Anion Transporter (OAT) Family[8,9]. Certain classes of drugs are transported across cell membranes by OATPs, notably in the liver and kidney. Oxidative phosphorylation transports compounds into hepatocytes for biotransformation by OATP. The Oligopeptide Transporters (OPTs or PEPTs) comprise a small gene family whose products transport substrates synthesized from amino acids including small peptides, secondary amino acids that can complex with metals, and the modified tripeptide glutathione.

Ochratoxin A (OTA) will destroy proximal tubule function and cause kidney damage in rats through increased anion conductance using the OATPs and PEPTs protein. Treat wild type and OATPs or PEPTs CRISPR KO rats with

increasing amounts of OTA for various durations and then use confocal microscopy to examine kidney function or tumors with formalin-fixed and paraffin-embedded mouse kidney slices[10].

2. Material and Methods

2.1 *OTA treatment*

1 mg of OTA was dissolved in 1 mL of absolute ethanol to obtain the stock solution (Sigma, >98% pure). The OTA stock solution was air dried before being resuspended in sterile culture medium containing 10% FCS and no antibiotics, which can prevent ethanol solution when experimenting the treatment. OTA concentrations between 1 nM and 100 mM were then applied for 24 to 72 hours to astrocytes. Treat wild type and OATPs or PEPTs CRISPR KO rats with increasing amounts of OTA for various durations and then use confocal microscopy to examine kidney function or tumors with formalin-fixed and paraffin-embedded mouse kidney slices[8,9].

2.2 *Examination and Checkpoint of the effect of OTA on glutamate clearance*

Clearances were normalized using the Folin procedure to determine the protein content in wells. Also performed recovery experiments. During the lab, astrocytes are treated with OTA for seventy two hours before the substance is changed and the cells are allowed to restore for 24–72 hours in serum-free medium. We omitted serum so that we would not generate new cells that would not have been exposed to OTA during the experiment[11]. We also conducted recovery experiments. As part of such experiments, astrocytes were first treated with OTA for 72 hours before changing the medium to serum-free media for 24–72 hours. To prevent cell division that could have affected the experiment, serum Serum was voluntarily omitted from the recovery medium to avoid generating new cells that were not exposed to OTA and potentially affected the experiment.

2.3 *Confocal microscopy*

Astrocytes seeded onto glass coverslips were immunostained and confocal microscopically examined. The coverslips were washed with PBS once after OTA treatment and fixed with 4% PFA in PBS for 15 minutes at room temperature. After threIn PBS supplemented with Triton X100 (0.1% final) and BSA (2% final), cells were permeabilized and blocked for 30 minutes.ee washes with PBS, astrocytes were double labelin PBS, astrocytes were double-labeled with primary rabbit anti-GFAP antibodies (Sigma, at a 1:100 dilution), and primary guinea pig anti-GLAST/GLLT-1 antibodies (Chemicon, at a 1:1000 dilution) and at room temperature for 1 hour.antibodies were detected with specifiWe detected primary antibodies with specific secondary antibodies (Invitrogen, used at a 1:200 dilution) conjugated either to AlexaFluor 488 for secondary antibodies directed against guinea pig antibodies (GLAST and GLT-1) or to (GFAP). Incubation with secondary antibodies lasted 1 h and took place at room temperature in the dark. Secondary antibodies were incubated at room temperature for 1 hour[12].

2.4 *Table possible results analysis*

Due to I don't have enough time, apparatus and materials to do such experiment, I design a table which contains 16 reasonable possible results of the experiment, and do some analysis to see whether these possible results support or not my hypothesis. It will have anion conductance and kidney damage by confocal microscopy. The detected substance in the table will be decreased conductance after OTA treatment in OATP+PETP WT cells, decreased conductance after OTA treatment in OATP+PETP KO cells, decreased conductance after OTA treatment in PETP KO cells, decreased conductance after OTA treatment in OATP KO cells and kidney damage markers after OTA treatment. The table will show plus sign or minus sign. Plus sign means that an increase of detecting substance compared with NC groups. Minus sign means that a decrease of detecting substance compared with NC groups. If a result contains only add sign, it means that the result is totally support my hypothesis. On the other hand, if a result contains only negative signs, it means that the result is totally oppose to my hypothesis. In other cases, if the result contains both puls signs and minus signs, it means that the result is partially support my result.

3. Results

Table 1. Detected Substance

Method	Detected substance	Result 1	Result 2	Result 3	Result 4	Result 5	Result 6	Result 7	Result 8	Result 9	Result 10	Result 11	Result 12	Result 13	Result 14	Result 15	Result 16
anion conductance	decreased conductance after OTA treatment in OATP+PETP WT cells	+	+	+	+	+	-	-	-	-	-	-	-	-	+	-	+
anion conductance	decreased conductance after OTA treatment in OATP+PETP KO cells	+	+	+	+	-	-	-	-	-	+	+	-	-	-	+	-
anion conductance	decreased conductance after OTA treatment in PETP KO cells	+	+	+	-	-	-	-	-	+	+	-	+	-	+	-	-
anion conductance	decreased conductance after OTA treatment in OATP KO cells	+	+	-	-	-	-	-	+	+	+	-	-	+	-	+	-
kidney damage by confocal microscopy	kidney damage markers after OTA treatment	+	-	-	-	-	-	+	+	+	+	-	-	-	+	-	+
Relation to the hypothesis	Totally support	Partially support	Partially support	Partially support	Partially support	Totally oppose	Partially support	Partially support	Partially support	Partially support	Partially support	Partially support	Partially support	Partially support	Partially support	Partially support	Partially support

Note. "+" represents an increase of detecting substance compared with NC groups. "-" represents a decrease of detecting substance compared with NC groups.

As shown in the table 1, there are 16 possible results below, which concluded whether the extent of the possible results support or refute the hypothesis.

Possible result 1: All the detected substance of anion substance and kidney damage by confocal microscopy will be positive.

Possible result 2: All the detected substance of anion substance will be positive but kidney damage by confocal microscopy will be negative.

Possible result 3: Decreased conductance after OTA treatment in OATP KO cells and kidney damage by confocal microscopy will be negative. Other will be positive.

Possible result 4: Decreased conductance after OTA

treatment in OATP+PETP WT cells and decreased conductance after OTA treatment in OATP+PETP KO cells will be positive. Other will be negative.
Possible result 5: Only decreased conductance after OTA treatment in OATP+PETP WT cells will be positive. Other will be negative.
Possible result 6: All the detected substance of anion substance and kidney damage by confocal microscopy will be negative.
Possible result 7: Only kidney damage markers after OTA treatment will be positive, other will be negative.
Possible result 8: Kidney damage markers after OTA treatment and decreased conductance after OTA treatment in OATP KO cells will be positive, other will be negative.
Possible result 9: Decreased conductance after OTA treatment in OATP+PETP WT cells and decreased conductance after OTA treatment in OATP+PETP KO cells will be negative. Other will be positive.
Possible result 10: Only decreased conductance after OTA treatment in OATP+PETP WT cells will be negative. Other will be positive.
Possible result 11: Only decreased conductance after OTA treatment in OATP+PETP KO cells will be negative. Other will be positive.
Possible result 12: Only decreased conductance after OTA treatment in OATP+PETP KO cells will be positive. Other will be negative.
Possible result 13: Only decreased conductance after OTA treatment in OATP KO cells will be positive. Other will be negative.
Possible result 14: Decreased conductance after OTA treatment in OATP KO cells and decreased conductance after OTA treatment in OATP+PETP KO cells will be negative. Other will be positive.
Possible result 15: Decreased conductance after OTA treatment in OATP KO cells and decreased conductance after OTA treatment in OATP+PETP KO cells will be positive. Other will be negative.
Possible result 16: Decreased conductance after OTA treatment in OATP+PETP WT cells and kidney damage markers after OTA treatment will be positive. Other will be negative.

4. Discussion

The effects of OTA on neurons have been independently demonstrated in numerous in vitro and in vivo studies. According to Table 1, during all the possible results, the possible results 1 best support the experimental hypothesis, which demonstrates all the detected substance will be positive.
And possible results 6 will reject the experimental hypothesis completely, which means that all the detected substance will be negative. Other 14 possible results will support and reject partially. According to the chart, result 1 shows OTA did affect kidney by increasing anion conductance using the OATPs and PEPTs protein, OTA destroyed the proximal tubule function in rats and caused kidney damage. Also treat wild type and OATPs or PEPTs CRISPR KO rats with increasing amounts of OTA for various durations and then use confocal microscopy to examine kidney function or tumors. In contrast, result 6 shows OAT substance have no influence to the OATPs and PEPTs protein and OATPs or PEPTs CRISPR KO rats. Thus will have no impact to the kidney function. Other result demonstrates a partly support to the hypothesis such as only OATPs protein is affected but PEPTs protein not or OATPs rat is affected but other is not affected. During the experiment, there are some measurement and substance may cause some error or deviation, but the problem is small and will not affect the results generally. Due to not enough time and apparatus to do such experiments, the possible results may be inaccurate to predict the ochratoxin behaviors and action, so this is a main possible error in the experiment. OTA has a direct impact on neurons and protein, but there is little information available on how it affects astrocytic viability and function. These pioneering studies did not evaluate whether OTA affects extracellular glutamate reuptake by astrocytes, regardless of how instructive they were. Astrocyte cultures enriched with OTA were examined in the present study to see if it affected OATPs and PEPTs protein. A physiological relevance of OTA's effect on OATPs and PEPTs is questionable because OTA directly affects protein viability. In addition to inhibiting OATP and PEPT viability, the concentrations of OTA that are able to cause renal disease also appear to reduce renal function. Accordingly, both of these effects of OTA could contribute to the destruction of proximal tubular structure and can cause kidney damage in rats by increasing anion conductance through the OATP and PEPT proteins[5-7] In light of the fact that OTA directly affects neuronal viability, one may ask if the effects of OTA on glutamate uptake by astrocytes are physiologically meaningful. In Table 1, OTA values are summarized for viability of KO and WT cells. Our results of OTA inhibiting OATP activity in wildtype rat astrocytes were compared with those showing OTA inhibiting neuronal viability in wildtype rats (Table 1) to assess their relevance.

5. Conclusion

The reabsorption and secretion of OTA by the renal tubule through the OAT system may play an essential role in OTA

accumulation and nephrotoxicity based on table 1 results. On the other hand, it is essential to consider the impact of albumin binding on transport in vitro when considering the clearance of OTA in vivo. By understanding the molecular mechanism of renal OTA transport, we can develop possible strategies for preventing OTA-induced renal damage. Our results demonstrate that the OATPs protein and the PEPTs protein could be used to destroy the proximal tubule by using the anion conductance of the OTA.

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