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Ochratoxin A induces nephrotoxicity and immunotoxicity through different MAPK signaling pathways in PK15 cells and porcine primary splenocytes

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HIGHLIGHTS

- Ochratoxin A induced nephrotoxicity and immunotoxicity in two kinds of cells.
- OTA induced p38 and ERK1/2 phosphorylation in two kinds of cells.
- Knock-down of p38 instead of ERK1/2 eliminated the OTA-induced nephrotoxicity in PK15 cells.
- Knock-down of ERK1/2 instead of p38 eliminated the OTA-induced immunotoxicity in porcine primary splenocytes.
- OTA induced nephrotoxicity and immunotoxicity through different MAPK signaling pathways.

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ABSTRACT

Ochratoxin A (OTA) is reported to be a potent nephrotoxin and immunotoxin in animals and humans. However, the mechanisms underlying OTA toxicity have not been clearly determined until now. Toxicity of OTA and its mechanism was investigated in PK15 cells and in porcine primary splenocytes. The results showed that OTA at 2.0–8.0 μg/mL for 24 h induced cytotoxicity and apoptosis in a dose-dependent manner in PK 15 cells. OTA at 0.5–4.0 μg/mL for 24 h induced cytotoxicity and apoptosis in a dose-dependent manner in porcine primary splenocytes. In addition, OTA induced p38 and ERK1/2 phosphorylation both in PK15 cells and porcine primary splenocytes. Knock-down of p38 instead of ERK by their specific siRNA significantly eliminated the nephrotoxicity induced by OTA. Contrary, knock-down of ERK1/2 instead of p38 by their specific siRNA significantly eliminated the immunotoxicity induced by OTA. The observed effects indicate that OTA induced nephrotoxicity by p38 signaling pathway in PK15 cells and immunotoxicity by ERK signaling pathway in porcine primary splenocytes.

1. Introduction

Ochratoxins, discovered in 1965, were the first major group of mycotoxins identified after the discovery of aflatoxins and are the secondary fungal metabolites of *Aspergillus* and *Penicillium* (Pitt, 1987; Creppy, 1999). Ochratoxin A (OTA), is the most prevalent and one of the most abundant mycotoxins. OTA, a world-wide mycotoxin, occurs in food and feeds under certain conditions and then accumulate in the meat of animals and human blood (Erkekoglu et al., 2010). The primary target organ of OTA is kidney, and OTA acts as a potent nephrotoxin in pigs. Moreover, due to its slow elimination from the body, OTA intake can lead to prolong internal exposure. In addition to being nephrotoxin, OTA is also an immunotoxin, affecting both humoral and cell-mediated immunity (Dehelean et al., 2011). OTA induced the decrease of growth performance, increased veterinary care costs, and decreased immunity in animals, which could result in enormous economic losses to the swine industry (Zain, 2011).

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The mechanisms underlying OTA toxicity have not been clearly determined. However, the oxidative stress induced by OTA could partly explain the mechanisms of OTA toxicity (Liu et al., 2012; Ciarcia et al., 2015). Yang et al. found that OTA treatment led to oxidative stress and apoptosis in human embryonic kidney cells (Yang et al., 2014a), and N-acetylcysteine (NAC) pretreatment protected the cells from OTA-induced damage (Yang et al., 2014b). Klaric et al. showed that OTA induced cytotoxicity and oxidative stress in porcine kidney 15 (PK15) cells (Klarić et al., 2008). Gan et al. found that OTA treatment led to oxidative stress and cytotoxicity in PK15 cells, and NAC pretreatment protected the PK15 cells from damage and buthionine sulfoximine pretreatment enhanced the OTA-induced cell damage (Gan et al., 2015). Periasamy et al. reported that OTA impaired immune function via oxidative stress in lymphocytes (Wang et al., 2009; Periasamy et al., 2016). In addition, oxidative stress could activate MAPK signal pathways (Gan et al., 2014b). However, there are few reports on the relationship of OTA and mitogen-activated protein kinase (MAPK) signaling pathway in pigs.

MAPK signal pathway, including p38, extracellular signal-regulated kinases (ERK) and c-Jun N-terminal kinase (JNK), are members of a ubiquitous protein serine/threonine kinase family (Johnson and Lapadat, 2002), participating in intracellular signaling during proliferation, differentiation, cellular stress responses, and apoptosis (Chang and Karin, 2001). It has been reported that MAPK signal pathway also plays a role in the mechanism of OTA toxicity. Wang et al. found that ERK and p38 MAPK signaling pathways play important roles in the regulation of OTA-induced G2 arrest in human gastric epithelium cells (Wang et al., 2012). Özcan et al. reported that OTA induced sustained activation of ERK1/2 signaling pathways in a dose- and time-dependent manner in human proximal tubule HK-2 cells. Further, chemical inhibition of ERK1/2 activation increased cell viability and decreased apoptosis in OTA-treated cells (Ozcan et al., 2015). Lada et al. showed that OTA could differentially activate ERK, JNK and p38 signal pathway in PK15 cells, and specific inhibitors of JNK and p38 increased cell viability measured by methyl thiazolyl tetrazolium (MTT) (Rumora et al., 2014). These reports clearly suggested that OTA toxicity is related to MAPK signal pathway. However, to date, there are no reports about the relationships between OTA-induced nephrotoxicity and immunotoxicity and MAPK signal pathways.

The objectives of this article were to study the OTA-induced toxicity in PK15 cells and porcine primary splenocytes and to investigate the roles of p38 and ERK1/2 MAPK signal pathway in OTA-induced nephrotoxicity and immunotoxicity.

2. Materials and methods

2.1. PK15 cells and porcine primary splenocytes culture and OTA preparation

The PK15 cell line was obtained from the China Institute of Veterinary Drug Control. The PK15 cells were maintained in Dulbecco's minimal Eagle's medium (DMEM, Invitrogen, USA) supplemented with 4% heat-inactivated fetal bovine serum (FBS), 1% penicillin, and 1% streptomycin at 37 °C in a humidified atmosphere of 5% CO_2.

The peripheral blood lymphocytes and splenocytes were separated by using a commercial Lymphocyte Separation Medium (Tianjin Hematology Institute) as described previously (Ren et al., 2012). The lymphocytes were cultured in Roswell Park Memorial Institute 1640 (RPMI-1640, Gibco, USA) medium supplemented with 4% heat-inactivated FBS, 1% penicillin, and 1% streptomycin at 37 °C in a humidified atmosphere of 5% CO_2.

OTA stock solution (2 mg/mL) used in the experiments was prepared by dissolving OTA in dimethyl sulfoxide (DMSO). Final concentrations of OTA were obtained by dilution in the culture medium. DMSO was added to cells without OTA treatment in the final concentration of 0.2%.

2.2. Cell viability assay in PK15 cells and porcine primary splenocytes

PK15 cells at a density of 4×10^3 cells/well or porcine primary splenocytes at a density of 5×10^5 cells/well with a stimulatory anti-pig-CD3 mAb were cultured in 96-well plates with corresponding treatment. Following this, MTT method is used for assessment of cell viability. Absorbance was measured at 490 nm and at a secondary wavelength of 650 nm. The results were expressed as percentage of control values.

2.3. Lactate dehydrogenase (LDH) release assay from PK15 cells and porcine primary splenocytes

For the assessment of LDH release, PK15 cells or porcine primary splenocytes with a stimulatory anti-pig-CD3 mAb were cultured in 12-well plates at a density of 8×10^4 cells/well or 8×10^5 cells/well with corresponding treatment. After the treatment, the culture medium was collected in 1.5 mL Eppendorf tubes and centrifuged at 12,000 rpm for 15 min at 4 °C. The supernatants were stored at −20 °C until analysis. LDH activity in the medium was determined as described previously (Dringen et al., 1998). One unit of enzyme activity was defined as equivalent to 1 μmol of reduced nicotinamide adenine dinucleotide oxidized per minute. The data were expressed as percentage of the control values.

2.4. Caspase-3 activity assay in PK15 cells and porcine primary splenocytes

For the assessment of caspase-3 activity, PK15 cells or porcine primary splenocytes with a stimulatory anti-pig-CD3 mAb were cultured in 6-well plates at a density of 2×10^5 cells/well or 1×10^6 cells/well with corresponding treatment. At the end of the treatment, caspase-3 activity in the cells was assessed using the colorimetric assay kit (KeyGEN, China) according to the manufacturer's instructions. Briefly, cells were washed twice with ice-cold phosphate buffered saline (PBS) and lysed by incubating in 100 μL of lysis buffer on ice for 30 min. The lysate was centrifuged at 12,000 rpm for 5 min at 4 °C and the supernatant was collected. Protein concentration in the supernatant was determined using the bicinchonininc acid (BCA) protein assay kit (Beyotime, China). Following this, 150 μg of each sample was incubated with caspase-3 substrate (20 mM Ac-DEVD-pNA) for 4 h at 37 °C in the dark. Then, the absorbance of the reaction mixture at 405 nm was measured using a microplate reader (Bio-RAD). Caspase-3 activity was calculated as optical density (OD) (inducer)/OD (negative control) and expressed as percentage of control values.

2.5. Annexin V binding assay by flow cytometry in PK15 cells and porcine primary splenocytes

For the assessment of apoptosis by annexin V staining, PK15 cells or porcine primary splenocytes with a stimulatory anti-pig-CD3 mAb were cultured in 6-well plates at a density of 2×10^5 cells/well or 1×10^6 cells/well with corresponding treatment and apoptosis was monitored by annexin V/propidium iodide (PI) (BD Pharmingen™) method as described previously (Yen et al., 2014) with minor modification. Briefly, after removing the culture medium, cells were washed two times with PBS, then resuspended