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PCV2 infection aggravates ochratoxin A-induced nephrotoxicity via autophagy involving p38 signaling pathway in vivo and in vitro☆

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ABSTRACT

Ochratoxin A (OTA) is reported to induce nephrotoxicity in animals and humans. Porcine circovirus type 2 (PCV2) could induce porcine dermatitis and nephropathy syndrome. To date, little is known whether virus infection aggravates mycotoxin-induced toxicity. This work aimed to study the effects of PCV2 infection on OTA-induced nephrotoxicity and its mechanism in vivo and vitro. The results in vivo showed that PCV2 infection aggravated OTA-induced poor growth performance, nephrotoxicity, p38 phosphorylation and autophagy as demonstrated by Atg5, LC3 II and p62 protein expressions in kidney of pigs. The results in vitro indicated that PCV2 infection significantly aggravated OTA-induced nephrotoxicity as demonstrated by cell viabilities, annexin V/PI binding and caspase 3 activities, and induced p38 phosphorylation and autophagy in PK15 cells. p38 inhibitor decreased Atg5 and LC3 protein expression induced by PCV2 infection and OTA combined treatment. Adding autophagy inhibitor 3-MA or CQ alleviated the aggravating effects of PCV2 infection on OTA-induced nephrotoxicity. Atg5-specific siRNA eliminated the aggravating effects of PCV2 infection on OTA-induced nephrotoxicity. Taken together, these data indicate that in vivo and in vitro PCV2 infection aggravated OTA-induced nephrotoxicity via p38-mediated autophagy.

1. Introduction

Ochratoxins produced by several species of *Aspergillus* and *Penicillium* families are the first major group of mycotoxins identified after aflatoxins (Creppy, 1999; Pitt, 1987). Ochratoxin A (OTA), one of the most abundant mycotoxin, presents in wide variety of foods and feeds (Palabiyik et al., 2013). Enzymes, especially proteolytic enzymes can degrade OTA (Hult et al., 1976; Upadhaya et al., 2012). The microbes in three forestomach compartments of ruminants degrade OTA, making ruminants insensitive to OTA. In contrast, after absorption, OTA degradation basically occurs in large intestine in non-ruminants, which makes non-ruminants, particularly pig more dangerous and sensitive to OTA among farmed animals (Hult et al., 1976). OTA contamination in pig feed and porcine nephrotoxicity are highly correlated (Bennett and Klich, 2003). OTA is a potent nephrotoxin and shown to be the main causative agent responsible for human Balkan endemic nephropathy (Cavin et al., 2009; Stefanovic and Polenakovic, 2009). It has been reported that accumulation of OTA and the severity of OTA-induced nephrotoxicity in animals and humans has been linked to animal management, the type of feed contamination, the dose, and so on (Ciarcia et al., 2016; Han et al., 2013). However, to date, whether presence of concurrent viral infections could affect OTA-induced nephrotoxicity has not been reported until now.

Porcine circovirus (PCV) is classified in the genus Circovirus of the Circoviridae family. Two genotypes of PCV have been recognized: the non-pathogenic PCV type 1 (PCV1) (Allan et al., 1995) and the pathogenic PCV type 2 (PCV2). PCV2 is the primary causative agent of porcine circovirus-associated disease (PCVAD), including porcine dermatitis and nephropathy syndrome (PDNS)

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(An et al., 2007; Rosell et al., 2000). PDNS was first described in the UK in 1993 (Smith et al., 1993) and the organs of frequent involvement are the kidneys and skin. In most affected animals, enlarged and pale kidneys with cortical petechiae are a prominent finding (Rosell et al., 2000). However, the precise cause of PDNS remain(s) elusive at this time (Opriessnig and Langohr, 2013). Our previous study indicated that OTA treatment could promote PCV2 infection (Gan et al., 2014). In contrast, whether PCV2 infection could aggravate OTA-induced nephrotoxicity remains unclear.

Since OTA has been reported to induce nephrotoxicity and PCV2 infection could induce kidney injury in pigs. Therefore, we hypothesize that: (i) PCV2 infection aggravates OTA-induced nephrotoxicity, (ii) PCV2 infection is a trigger factor for OTA-induced nephrotoxicity, (iii) PCV2 infection impacts OTA-induced nephrotoxicity via autophagy involving p38 signaling pathway. Thus, the aims of the current work were to study the effect of PCV2 infection on OTA-induced nephrotoxicity in vivo and vitro and the autophagy mechanism involved by using pigs and PK15 cells as the models.

2. Material and methods

The detailed material and methods descriptions were provided in supplemental file.

3. Results

3.1. PCV2 infection aggravates OTA-induced nephrotoxicity in pigs

The histopathological changes and TUNEL-stained cells of kidneys and levels of serum creatinine and urea in piglets are shown in Fig. 1. There were hyperchromatic nuclei and cytoplasm, nuclei atrophy, necrosis and exfoliation in proximal tubules epithelial cells of piglets in PCV2 infection group and OTA at 400 μg/kg fed group (Fig. 1A). PCV2 infection aggravated lesions of kidney induced by OTA (Fig. 1A). PCV2 infection (Fig. 1B)or OTA feeding (Fig. 1B) significantly increased apoptosis of kidney as demonstrated by TUNEL-stained cells. PCV2 infection aggravated OTA-induced apoptosis (Fig. 1B). In addition, PCV2 infection or OTA treatment alone increased the serum creatinine levels on Day 21 and 42 compared with the Con group. PCV2 infection increased changes of creatinine (Fig. 1C) and urea (Fig. 1D) levels induced by OTA. These results suggest that PCV2 infection aggravates OTA-induced nephrotoxicity of pigs.

3.2. PCV2 infection aggravates OTA-induced autophagy in kidney of pigs

The protein levels of p38, pp38, p62, LC3 II and Atg5 in the kidney of piglets from four treatment groups are shown in Supplemental Fig. 2 in supplemental file. PCV2 infection or OTA treatment significantly increased pp38, Atg5 and LC3 II protein levels ($P < 0.05$) and decreased p62 protein levels compared with control group. Further, PCV2 infection promoted the increases of pp38, Atg5 and LC3 II protein levels compared with OTA group. However, total forms of p38 remained unaltered in the three experiment groups compared with Con group. These results suggest that PCV2 infection aggravates the autophagy induced by OTA in kidney of pigs.

3.3. PCV2 infection aggravates OTA-induced nephrotoxicity in PK15 cells

To further assess whether potential effects of PCV2 infection on OTA-induced nephrotoxicity, we examined the effects of PCV2 infection (MOI = 1) on cytotoxicity and apoptosis induced by OTA at 2.0 and 2.5 μg/mL for 48 h. As shown in Fig. 2, OTA at 2.0 and 2.5 μg/mL had no effects on PCV2 infection (Fig. 2A). PCV2 infection alone had no effects on cell viabilities, but increased caspase 3 activity and Annexin V/PI bing in PK15 cells. OTA at 2.0 and 2.5 μg/mL decreased cell viabilities (Fig. 2B) and increased caspase 3 activity (Fig. 2C) and Annexin V/PI bing (Fig. 2D and E), compared with the Con group. PCV2 infection aggravated the OTA-induced nephrotoxicity by decreasing cell viabilities, and increasing Annexin V/PI binding and caspase 3 activities ($P < 0.05$). These results suggest that PCV2 infection aggravates OTA-induced nephrotoxicity in PK15 cells.

3.4. PCV2 infection aggravates OTA-induced autophagy in PK15 cells

To examine whether the aggravating effects of PCV2 infection on OTA-induced nephrotoxicity are related to autophagy mechanism, GFP-LC3, Atg5, LC3 II and p62 proteins expressions are measured. PK15 cells were infected with or without PCV2 infection for 24 h, then incubated with or without OTA at 2.0 μg/mL for an additional 48 h. OTA treatment alone increased GFP-LC3 expressions (Fig. 3A), and decreased p62 expression (Fig. 3B and C) and increased Atg5 (Fig. 3B and D) and LC3 II expression (Fig. 3B and E) in PK15 cells ($P < 0.05$) compared with control group. PCV2 infection further increased LC3 and Atg5 expression and inhibited p62 expression induced by OTA (Fig. 3) ($P < 0.05$). These results suggest that PCV2 infection aggravates OTA-induced autophagy and the aggravating effects of PCV2 infection on OTA-induced nephrotoxicity may be related to its effects on autophagy.

3.5. P38 inhibitor weakens the autophagy induced by PCV2 infection and OTA treatment

We further explored whether p38 signaling pathway mediates the autophagy mechanism in the aggravating effects of PCV2 infection on OTA-induced nephrotoxicity in PK15 cells. Results are shown in supplemental file, PCV2 infection or OTA treatment increased expressions of LC3-II and Atg5 proteins and p38 phosphorylation. PCV2 infection enhanced the above changes induced by OTA. Adding p38 inhibitor (SB203580) weakened the increases of pp38, LC3-II and Atg5 induced by PCV2 infection and OTA treatment. These results suggest that p38 plays a role in the autophagy mechanism of the aggravating effects of PCV2 infection on OTA-induced nephrotoxicity in PK15 cells.

3.6. Autophagy mediates the aggravating effects of PCV2 infection on OTA-induced nephrotoxicity in PK15 cells

In order to verify the role of autophagy in the aggravating effects of PCV2 infection on OTA-induced nephrotoxicity, we used autophagy inhibitor (3-MA, CQ) and activator (rapamycin) to study its effects on the OTA-induced nephrotoxicity aggravated by PCV2 infection. As shown in Fig. 4, Autophagy inhibitor, 3-MA or CQ inhibited LC3 II expression and rapamycin promoted LC3 II expression induced by PCV2 infection and OTA combined treatment (Fig. 4D). Rapamycin exacerbated the effects of PCV2 infection on OTA-induced nephrotoxicity. 3-MA or CQ, alleviated the aggravating effects of PCV2 infection on OTA-induced nephrotoxicity as demonstrated by poor cell viabilities (Fig. 4A) and decreasing apoptosis (Fig. 4B, C, E). Further, to address whether Atg5 plays a role in the OTA-induced nephrotoxicity aggravated by PCV2 infection, Atg5-specific siRNA was used. Results are shown in Supplemental Fig. 5 in supplemental file and are similar to the above results. These results demonstrate that autophagy plays a key role in the aggravating effects of PCV2 infection on OTA-induced nephrotoxicity in PK15 cells.