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Effect of heme oxygenase-1 on ochratoxin A-induced nephrotoxicity in mice

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ABSTRACT

Heme oxygenase-1 (HO-1), a heme-degrading enzyme, is suggested to play an important role in kidney pathophysiology, mostly due to its anti-fibrotic, anti-apoptotic and anti-oxidant properties. One of the mycotoxin, ochratoxin A (OTA) was previously shown to affect HO-1 expression, however, the mechanisms of OTA-induced nephrotoxicity during HO-1 deficiency are unknown.

We have shown that OTA regulates the number of pro-fibrotic, pro-inflammatory, anti-oxidative and pro-apoptotic factors in HO-1 dependent manner, as the lack of HO-1 accelerates whereas the induction of HO-1 expression by cobalt protoporphyrin (CoPP) attenuates nephrotoxic effect of OTA. The down-regulation of the nuclear factor-erythroid-2- related factor 2 (Nrf2) transcription factor by OTA, observed in HO-1 knock-out animals, might be another mechanism of OTA toxicity. Moreover, HO-1 level and OTA treatment influences the expression of microRNAs. Namely, p53-regulated miR-34a and pro-fibrotic miR-21 were already increased in HO-1$^{-/-}$ kidneys and were further induced by OTA administration, whereas anti-fibrotic miR-29c was down-regulated by this mycotoxin.

Our study indicates that complex mechanisms of OTA nephrotoxicity may be partially overcome by HO-1 induction.

1. Introduction

Virtually all chronic kidney diseases lead to the tubulointerstitial fibrosis, a pathologic state characterized by accumulation of myofibroblasts, induction of epithelial-mesenchymal transition (EMT) and extracellular matrix (ECM) deposition in the interstitium, a condition associated with the failure of the organ's function. Transforming growth factor-β (TGF-β), a key pro-fibrotic cytokine, controls various cellular processes, including inflammation, proliferation, apoptosis and differentiation (reviewed in (Gewin and Zent, 2012)). Through the induction of target genes TGF-β promotes fibroblast survival and proliferation, induces EMT and leads to the deposition and remodeling of ECM. Some studies underline also TGF-β-independent pathways as mechanisms of tubulointerstitial fibrosis (Lavoz, 2012).

The involvement of heme oxygenase-1 (HO-1), the enzyme converting heme into biliverdin, carbon monoxide and iron ions in the protection against kidney diseases was suggested in different experimental settings (reviewed in (Jarmi and Agarwal, 2009)). Firstly demonstrated in a model of acute kidney injury (AKI) (Nath et al., 1992), then its protective effect was also shown in chronic kidney diseases. In unilateral ureteral obstruction (UUO), a commonly used model of kidney injury leading to tubulointerstitial fibrosis, HO-1 induction by hemin administration was demonstrated to reduce the progression of disease through modulating immune response, decreasing TGF-β expression and collagens deposition (Correa-Costa, 2010). Kim et al. (2006) underlined the role of apoptotic signaling in the protection against UUO, as they observed an increase in the level of Bcl-2 as well as the reduction in activation of caspase-3 when expression of HO-1 was increased by hemin. Not only hemin, but also other HO-1 inducer, cobalt protoporphyrin (CoPP) was able to attenuate nephropathy in rats (Iwai et al., 2008). However, it is important to remember, that level of HO-1 induction might be crucial for the cytoprotective effect of this enzyme. It has been suggested that too high induction of HO-1, possibly due to increased iron release may, in fact, be deleterious (Suttner and Dennery, 1999).

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Our in vitro studies performed on kidney epithelial cell line (Boesch-Saadatmandi et al., 2008; Stachurska et al., 2013) indicate the important nephroprotective role of HO-1 and its regulator, nuclear factor-erythroid-2-related factor 2 (Nrf2). We have shown that ochratoxin A (OTA), one of the possible factors responsible for Balkan endemic nephropathy and probable causative agent in the development of urinary tract tumors (reviewed in (Malir et al., 2016)), down-regulates Nrf2/HO-1 signaling in porcine proximal tubular epithelial cells (Boesch-Saadatmandi et al., 2008). The de-regulation of Nrf2 pathway in OTA-mediated toxicity was also demonstrated by other groups in both in vitro and in vivo models (Cavin et al., 2009; Cavin et al., 2007; Marin-Kuan et al., 2006). In our hands, adenoviral overexpression of Nrf2 or HO-1 halted OTA-mediated induction of TGF-β as well as reactive oxygen species production (Stachurska et al., 2013). Crucial observations clarifying the role of HO-1 in fibrotic process comes from HO-1$^{-/-}$ animal models (Kie et al., 2008; Kovtunovych et al., 2010; Poss and Tonegawa, 1997) as well as from two patients with HO-1 deficiency described till now (Yachie et al., 1999; Radhakrishnan et al., 2011). In mice, lack of HO-1 leads to anemia and accumulation of renal iron causing tissue injury and chronic inflammation (Kovtunovych et al., 2010; Poss and Tonegawa, 1997). Accordingly, in HO-1 knockout mice more severe manifestations of kidney disease have been noticed after UUO including greater fibrosis and more extensive EMT than in wild-type mice (Kie et al., 2008). In human HO-1 deficiency similar phenotypic symptoms, mostly renal tubulointerstitial injury with inflammatory cell infiltration and fibrosis, have been observed (Yachie et al., 1999; Radhakrishnan et al., 2011).

A growing body of evidence suggests the involvement of specific microRNAs in kidney diseases (reviewed in: (Patel and Noureddine, 2012; Trionfini et al., 2015)). The pro-fibrotic microRNAs, like miR-21, contribute to fibrogenesis through positive regulation of ECM and could therefore be candidate targets for anti-fibrotic therapies (Zarjou et al., 2011a). Moreover, expression of miR-34a which is regulated by p53 signaling could be altered in kidney diseases including cisplatin nephrotoxicity (Bhatt et al., 2010). On the other hand, the families of anti-fibrotic miR-29 and miR-200 have been shown to inhibit tissue fibrosis in different kidney diseases (reviewed in: (Patel and Noureddine, 2012)). Previously, we have demonstrated, that OTA may regulate the expression of specific microRNAs, small noncoding RNAs, which act as post-transcriptional regulators of gene expression and that this toxin affects the total pool of microRNAs in LLC-PK1 cells by altering the level of enzymes involved in the microRNAs biogenesis/processing (Stachurska et al., 2013). Then microRNA profiling was performed in rats after different doses of OTA (Dai et al., 2014) as well as the involvement of miR-29b in OTA-induced collagen formation has been described (Hennemeier et al., 2014).

In the present study, we found that OTA exerts nephrotoxic activities through modulation of the expression of fibrotic, apoptotic, anti-oxidant and inflammatory markers, as well as it affects the expression of specific microRNAs. Using an unique, HO-1 knockout mouse model we have demonstrated that OTA acts in an HO-1-dependent way. In our hands, the lack of HO-1 augments a number of OTA-caused toxic effects, at least partially through Nrf2 inhibition. Accordingly, HO-1 induction in vivo attenuates OTA-induced nephrotoxicity, apparently through diminishing the expression of selected pro-fibrotic and inflammatory factors.

2. Materials and methods

2.1. Ethics statement

The study protocol was approved by the Institutional Animal Care and Use Committee at the Jagiellonian University. The animals were maintained under a constant 12 h dark/light cycle at an environmental temperature of 22 ± 2 °C and were provided with normal laboratory pellet diet and water *ad libitum.*

2.2. Cell culture and incubation experiments

LLC-PK1 cell line, an established epithelial cell line derived from porcine renal cortex, was kindly supplied by Prof. Gerald Rimbach (Institute of Human Nutrition and Food Science, Christian Albrechts University Kiel, Germany). The cells were cultured in high glucose DMEM medium, supplemented with 10% FBS, streptomycin (100 μg/ml) and penicillin (100 U/ml), and kept under standard conditions (37 °C, 5% CO2). OTA was prepared in methanol and 25 μM OTA was used for stimulation. PD98059, a specific ERK1/2 inhibitor, was dissolved in DMSO. Cells were prestimulated with 10 μM PD98059 for 30 min followed by OTA treatment for 6 h. Then RNA was isolated and gene expression was checked by real-time PCR.

2.3. Animals

Experiments were performed on C57BL/6 × FVB mice with normal level of HO-1 (HO-1$^{+/+}$), and on littermate mice lacking both HO-1 alleles (HO-1$^{-/-}$) of both sexes. The mice were generated from the HO-1$^{+/-}$ breeding pairs kindly gifted by Dr. Anupam Agarwal (Birmingham, AL, USA). Animals were genotyped three weeks after birth by PCR using tail DNA as previously described (Poss and Tonegawa, 1997).

2.4. Experimental procedure

6-month old HO-1$^{+/+}$ and HO-1$^{-/-}$ mice were injected intraperitoneally (i.p.) with 2.5 mg/kg bw OTA (Sigma-Aldrich) dissolved in 0.1 M $NaHCO_3$ every other day for 20 days. Control mice were injected with the solvent (data presented in Figs. 1 A, B, E; 2 ; 3 ; 5 A–G, H; 6 A–E; 7, 8C). In the other set of experiments, which aimed to check the protective effect of HO-1 induction on kidney fibrosis, 4 groups of wild-type mice have been established: i) control mice (receiving solvents: 20 mM NaOH and 0.1 M $NaHCO_3$); ii) OTA-injected animals (2.5 mg/kg bw in 0.1 M $NaHCO_3$); iii) CoPP-injected mice (10 mg/kg bw CoPP in 20 mM NaOH); iv) CoPP and OTA-injected animals (results presented in Figs. 1C–D; 4 ; 5H, I; 6F–H). CoPP, the HO-1 inducer, was administered i.p. once a week starting from the day 1. Starting from the day 2, mice were injected with OTA every second day. In the last set of experiments, CoPP was administered in the same dose but more frequently (every 4 days) (Fig. 9). At the end of experiments blood was collected for serum preparation as well as kidney fragments have been preserved for the downstream analysis.

2.5. Reverse transcription–Polymerase chain reaction and real-time PCR

Total RNA was isolated by lysis of kidney fragments in 1 ml of QIAzol Total RNA Isolation Reagent using TissueLyser (Qiagen) and then the modified Chomczynski and Sacchi (Chomczynski and Sacchi, 1987) RNA isolation protocol was applied. Reverse transcription was performed using SuperScript polymerase (Invitrogen) or NCodeTM VILO™ miRNA cDNA Synthesis Kit (Invitrogen) as described earlier (Stachurska et al., 2013). Quantitative real-time PCR was done by the use of StepOnePlus™ Real-Time PCR System (Applied Biosystems). The reaction mixture contained SYBR Green PCR Master Mix (Sigma-Aldrich), reverse and forward primers and 50 ng of cDNA. Relative gene expression level was calculated as $2^{-\Delta C_T}$, where ΔC_T is defined as a difference between C_T values