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Original Research Article

Evodiamine attenuates cadmium-induced nephrotoxicity through activation of Nrf2/HO-1 pathway

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Abstract

Purpose: *To investigate the protective role of evodiamine, a naturally occurring anti-inflammatory, antioxidant, and anti-apoptotic compound, against cadmium-induced cytotoxicity in proximal tubular cells (human kidney 2; HK-2).*
Methods: *HK-2 cells were treated with different concentrations of evodiamine (5, 20, 50 μM) for 2 h and then incubated with 40 μM cadmium chloride for another 24 h. Cell viability and apoptosis were evaluated using thiazolyl blue tetrazolium bromide (MTT) and flow cytometry, respectively. Oxidative stress was assayed by measuring the levels of malonaldehyde (MDA), superoxide dismutase (SOD), glutathione (GSH) and glutathione peroxidase (GSH-PX).*
Results: *Cadmium chloride treatment in HK-2 cells significantly reduced cell viability ($p < 0.01$) and increased apoptosis compared to the control. Evodiamine pretreatment attenuated the cadmium chloride-provoked decrease in cell viability and increase in apoptosis. Evodiamine also decreased expression of cleaved caspase-3 and cleaved caspase-9 in HK-2 cells. Cadmium chloride exposure provoked kidney injury, as evidenced by increased MDA levels and decreased SOD, GSH, and GSH-PX levels. Pretreatment with evodiamine ameliorated kidney injury, as shown by decreased MDA expression and increased SOD, GSH, and GSH-PX expression. Evodiamine exposure significantly enhanced protein expression of nuclear factor erythropoietin-2-related factor 2 (Nrf2) and heme oxygenase 1 (HO-1).*
Conclusion: *Evodiamine exerts an anti-apoptotic and anti-oxidative effect against cadmium chloride-induced nephrotoxicity via Nrf2/HO-1 pathway activation. These findings represent a potential therapeutic strategy for cadmium-provoked nephrotoxicity.*

Keywords: *Evodiamine, Cadmium, Nephrotoxicity, Nrf2/HO-1, Apoptosis, Oxidative stress*

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INTRODUCTION

Cadmium is one of the most cumulative human poisons with a half-life of over 10 years [1]. Renal proximal tubule epithelial cells absorb the cadmium. Ultimately, about 50 % of cadmium accumulates in the kidney, induces metabolic disorders, organ damage, and nephrotoxicity [2].

Therefore, therapeutic agents that protect against cadmium-provoked nephrotoxicity are needed.

Cadmium accumulation enhances free radical production through repression of the cellular redox defense system and production of redox-active metals [3]. Free radicals trigger oxidative stress that can lead to tissue damage, such as acute and chronic kidney injury [4]. Therefore, antioxidants may play a role in attenuating cadmium-provoked nephrotoxicity.

The natural indole alkaloid, evodiamine, is isolated from *Evodia rutaecarpa*. Critically, evodiamine has analgesic, anti-obesity, anti-inflammatory, anti-atherosclerosis, neuroprotective, anti-cancer, and anti-oxidative effects [5]. For example, evodiamine has an anti-proliferative effect against breast cancer cells [6] and promotes blood-brain barrier permeability in cerebral ischemia [7]. Ischemia reperfusion-induced renal injury is alleviated by the antiapoptotic, anti-inflammatory, and antioxidative properties of evodiamine [8]. Evodiamine prevents lipopolysaccharide-provoked production of reactive oxygen species and inflammatory response during acute kidney injury [9]. Whether evodiamine inhibits oxidative stress and apoptosis during cadmium-provoked nephrontoxicity has yet to be determined.

In the present study, HK-2 cells were pretreated with evodiamine and then incubated with cadmium chloride to stimulate cytotoxicity. The effects of evodiamine on cadmium chloride-induced apoptosis and oxidative stress were then evaluated.

EXPERIMENTAL

Cell culture and treatment

HK-2 cells (American Type Culture Collection, Manassas, VA, USA), were cultured in Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (Invitrogen, Carlsbad, CA, USA) containing 10 % fetal bovine serum (Sigma-Aldrich, St. Louis, MO, USA) in a 37 °C incubator. The HK-2 cells were incubated with different concentrations of evodiamine (5, 20, and 50 μM) (Sigma-Aldrich) for 2 h and then incubated with 40 μM cadmium chloride (Sigma-Aldrich) for another 24 hours before the functional assays.

Determination of cell viability and apoptosis

HK-2 cells were seeded into a 96-well plate for 24 hours and incubated with 20 μL MTT solution (5 mg/mL; Sigma-Aldrich). Four hours later, the culture medium was removed and cells were incubated with dimethyl sulfoxide. Optical density at 490 nm was measured by microplate spectrophotometer (ELx800, Bio-TEK, Winooski, VT, USA). HK-2 cells were harvested and resuspended in binding buffer.

The cells were then incubated with propidium iodide and Annexin V-fluorescein isothiocyanate according to the Annexin V-Fluorescein Isothiocyanate Apoptosis detection kit instructions (Calbiochem, San Diego, CA, USA). The apoptotic ratio of HK-2 cells was analyzed using flow cytometry (Attune, Life Technologies, Darmstadt, Germany).

Biochemical analysis

HK-2 cells were harvested and lysed in Cell Lysis Buffer (Sigma Aldrich). After suspending in phosphate-buffered saline, protein concentrations were measured using Bicinchoninic Acid Protein Assay kit (Beyotime Institute of Biotechnology, Shanghai, China). Levels of malonaldehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), and glutathione peroxidase (GSH-PX) were determined using commercial assay kits (Sigma Aldrich).

Western blot

HK-2 cells were lysed 24 h after cadmium chloride exposure. Protein samples were then separated using electrophoresis. The samples were electroblotted onto nitrocellulose membranes, and the membranes were blocked in 5 % skim milk. The membranes were incubated with primary antibodies against cleaved caspase-3 and 9 (1 : 2000; Abcam, Cambridge, MA, USA), nuclear factor erythropoietin-2-related factor 2 (Nrf2) and heme oxygenase 1 (HO-1) (1 : 3000; Cell Signaling Technology, Danvers, MA, USA), and GAPDH (1 : 4000; Abcam) and then incubated with horseradish peroxidase-linked secondary antibody (1 : 5000; Cell Signaling Technology). Signal was detected using the enhanced chemiluminescence detection kit (GE Healthcare, Chicago, IL, USA).

Statistical analysis

Data are expressed as mean ± standard error of mean. Statistical differences were determined by Student's t-test or by one-way analysis of variance. The statistical difference was assessed with a p-value of < 0.05.

RESULTS

Evodiamine mitigated cadmium chloride-induced cytotoxicity in HK-2 cells

To investigate the effect of evodiamine on cadmium-induced nephrotoxicity, HK-2 cells were pretreated with evodiamine (Figure 1 A) and then incubated with cadmium chloride. Data from MTT and flow cytometry showed that cadmium chloride decreased cell viability (Figure 1 B) and increased apoptosis (Figure 2 A) in HK-2 cells. However, preconditioning with evodiamine increased cell viability (Figure 1 B) and decreased cell apoptosis (Figure 2 A) in HK-2 cells. Moreover, evodiamine exposure attenuated the cadmium chloride-induced increase of cleaved caspase-3 and -9 (Figure 2B), suggesting that evodiamine demonstrated an anti-apoptotic effect against cadmium-induced nephrotoxicity.

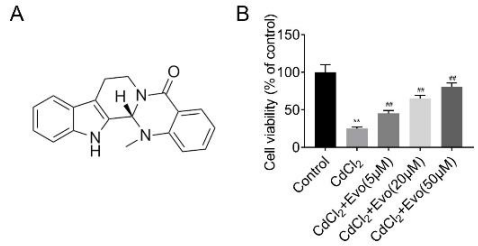

Figure 1: Evodiamine mitigated cadmium chloride-induced decreased cell viability in HK-2 cells. (A) Chemical structure of evodiamine. (B) Preconditioning with evodiamine dose-dependently attenuated decreased cell viability induced by cadmium chloride in HK-2 cells; **, ## $p < 0.01$

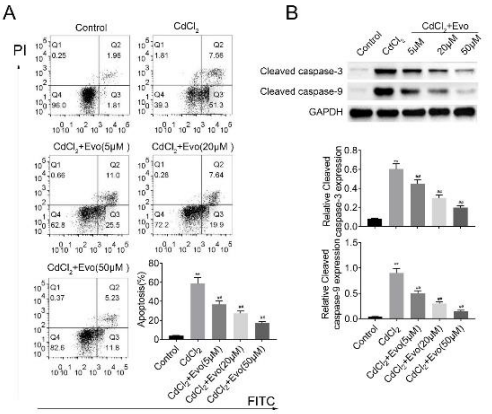

Figure 2: Evodiamine mitigated the cadmium chloride-induced increase in apoptosis in HK-2 cells. (A) Preconditioning with evodiamine attenuated cadmium chloride-induced increase of cell apoptosis in HK-2 cells. (B) Evodiamine treatment attenuated the cadmium chloride-induced expression of cleaved caspase-3 and -9 in HK-2. **, ## $p < 0.01$

Evodiamine mitigated cadmium chloride-induced promotion of oxidative stress in HK-2 cells

HK-2 cells under cadmium chloride exposure showed increased levels of MDA (Figure 3 A) and decreased levels of SOD (Figure 3 B), GSH (Figure 3 C), and GSH-PX (Figure 3 D). However, preconditioning with evodiamine reduced the level of MDA (Figure 3 A) and enhanced SOD, (Figure 3B), GSH (Figure 3C), and GSH-PX (Figure 3 D) levels in HK-2 cells. Together, these results suggest that evodiamine produced an anti-oxidative effect against cadmium-induced nephrotoxicity.

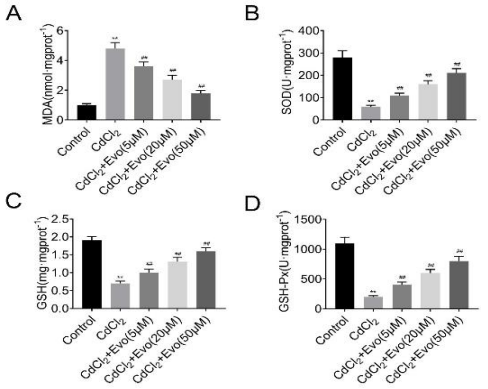

Figure 3: Evodiamine mitigated cadmium chloride-induced promotion of oxidative stress in HK-2 cells. (A) Preconditioning with evodiamine dose-dependently attenuated cadmium chloride-induced increase of MDA expression in HK-2 cells. (B) Preconditioning with evodiamine attenuated cadmium chloride-induced decrease of SOD expression in HK-2 cells. (C) Preconditioning with evodiamine attenuated cadmium chloride-induced decrease of GSH expression in HK-2 cells. (D) Preconditioning with evodiamine attenuated cadmium chloride-induced decrease of GSH-PX expression in HK-2 cells; **, ## $p < 0.01$

Evodiamine modulated the Nrf2 pathway in HK-2 cells under cadmium chloride exposure

We employed western blot analyses to determine whether evodiamine pretreatment regulated expression of members of the Nrf2 pathway, which is involved in regulation of oxidative stress. Results showed that protein expression of Nrf2 and HO-1 were enhanced in HK-2 under cadmium chloride exposure (Figure 4). Preconditioning with evodiamine produced higher protein expression of Nrf2 and HO-1 than that of HK-2 under cadmium chloride exposure alone (Figure 4). These results suggest that evodiamine promoted activation of the Nrf2 pathway to ameliorate cadmium-induced nephrotoxicity.

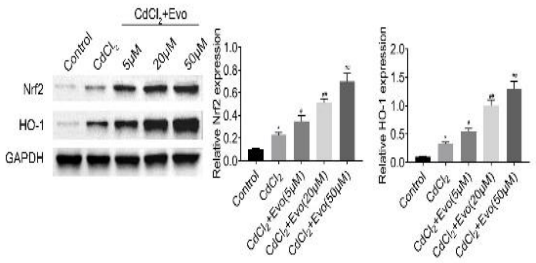

Figure 4: Evodiamine modulated expression of Nrf2 pathway members in HK-2 cells under cadmium chloride exposure. Preconditioning with evodiamine dose-dependently promoted cadmium chloride-induced expression of Nrf2 and HO-1 in HK-2 cells; *, # $p < 0.05$, ## $p < 0.01$

DISCUSSION

Cadmium accumulation in the proximal tubule of the nephrons leads to proximal tubular reabsorptive defect and, ultimately, nephrotoxicity through promotion of Fanconi syndrome and suppression of Na-K-ATPase activity [10]. Cadmium chloride exposure induces oxidation of dihydrorhodamine 123 and promotes reactive oxygen species in proximal tubule cell lines [10]. However, co-incubating proximal tubule cells with the thiol antioxidant, N-acetylcysteine, prevents oxidative damage [10]. Thus, employing antioxidants to prevent cadmium-induced nephrotoxicity is highly feasible.

Cadmium-induced cytotoxicity may promote proximal tubular cell apoptosis and, ultimately, result in human kidney damage [11]. Here, we demonstrated that cadmium chloride exposure provoked decreased cell viability and increased cell apoptosis in HK-2 cells. A previous study has shown that evodiamine seems to function as a pro-apoptotic factor to repress tumor progression [12]. Conversely, another study indicated that zymosan-induced cell apoptosis in the intestine and lung is attenuated by evodiamine [13]. Data in this study demonstrated the anti-apoptotic role of evodiamine in cadmium-treated HK-2 cells through down-regulation of cleaved caspase-3 and -9. The signaling pathways involved in the anti-apoptotic role of evodiamine on cadmium-induced nephrotoxicity merit further investigation.

Cadmium may induce kidney injury by increasing reactive oxygen species and MDA in addition to aggravating kidney apoptosis [14]. Evodiamine decreases levels of total oxidant status and increases total antioxidant status to mitigate ischemia reperfusion-induced kidney injury [8]. Furthermore, evodiamine treatment represses lipopolysaccharide-induced production of reactive oxygen species in *Rattus norvegicus* kidney cells [9]. The present study revealed that evodiamine exposure attenuated the cadmium chloride-induced increase in MDA levels and decrease in SOD, GSH, and GSH-PX levels in HK-2 cells. Together, these results suggest that evodiamine has an anti-oxidative effect against cadmium-induced nephrotoxicity.

After nuclear translocation, Nrf2 binds with the antioxidant response element of target genes, including HO-1, GSH-PX, and SOD, to negatively regulate oxidative stress [15]. Activation of the Nrf2 pathway contributes to the amelioration of renal injury [15]. Carnosic acid-induced promotion of Nrf2/HO-1 suppresses oxidative stress to alleviate cadmium-induced nephrotoxicity [16]. Moreover, Nrf2 pathway activation plays a protective role in cadmium-induced kidney cells apoptosis [11]. Intriguingly, a previous study showed that evodiamine activates Nrf2/HO-1 signaling in BV-2 cells [17]. This study also revealed that preconditioning with evodiamine enhances protein expression of Nrf2 and HO-1.

This study further suggests that evodiamine may ameliorate cadmium-induced nephrotoxicity through activation of the Nrf2/HO-1 pathway. In addition, cadmium-provoked inflammation contributed to nephrotoxicity [18], and evodiamine slowed nuclear factor kappa B-modulated inflammation during development of lipopolysaccharide-induced acute kidney injury [9]. Whether evodiamine exerts an anti-inflammatory effect against cadmium-induced nephrotoxicity needs to be investigated.

CONCLUSION

Evodiamine protects HT-2 cells from cadmium-induced nephrotoxicity through its anti-apoptotic and anti-oxidative properties. Evodiamine appeared to attenuate cadmium-induced toxicity by activating the Nrf2/HO-1 pathway. However, the effect of evodiamine *in vivo* should be investigated to validate its potential therapeutic role in cadmium-provoked nephrotoxicity.

DECLARATIONS

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Competing interests

The authors state that there are no conflicts of interest to disclose.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.
Zhichun Song and Wei Wang designed the study and supervised the data collection. Xiaoren Zhang and Hongsheng Yu analyzed and interpreted the data. Chunsheng Qu, Shu Dai and Xiaodong Wang prepared the manuscript for publication and reviewed the draft of the manuscript. All authors read and approved the manuscript.

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