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Protective effects of dietary luteolin against mercuric chloride-induced lung injury in mice: Involvement of AKT/Nrf2 and NF-κB pathways

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ABSTRACT

Food-derived compound luteolin possesses multiple pharmacological activities. Accordingly, we focused on exploring the protective effects of luteolin (100 mg/kg) against mercuric chloride ($HgCl_2$) (5 mg/kg) stimulated lung injury and the molecular mechanisms of lung protection effects in mouse. The influence of luteolin on histologic changes, oxidative stress, proinflammatory cytokine production, neutrophil activation, and apoptosis were assayed in $HgCl_2$-induced lung injury. Luteolin administration attenuated pulmonary histologic conditions and apoptotic change. The protective effects of luteolin might be attributed to the reduction of myeloperoxidase, inflammatory cytokines, malondialdehyde, and the increase of superoxide dismutase and glutathione. Luteolin promoted protein kinase B (AKT) phosphorylation and translocation of nuclear factor erythroid 2-related factor 2 (Nrf2) into nucleus, and inhibited activation of nuclear factor kappa B (NF-κB) in $HgCl_2$-induced lung injury. Taken together, dietary luteolin may be an effective candidate for treatment of $HgCl_2$-induced lung injury by preventing NF-κB activation and activating AKT/Nrf2 pathway.

1. Introduction

Mercury is a persistently toxic element that concentrates up the food chain (Mahbub et al., 2017a). However, mercury possesses many beneficial properties, and has been used in many fields, such as ingredient of many traditional medicines (Pal et al., 2014), pigment (Shephard, 2015), and so on. Mercury mobilization caused soil and water contamination (Mahbub et al., 2017b). To date, abundant reports have revealed that the toxicity of mercury in humans as a result of mercury contamination in fish, vegetables, and fruits (Rodríguez-Hernández et al., 2016; Llobet et al., 2003). In addition, a maximum safe exposure level of mercury has yet to be determined (Rasinger et al., 2017). Therefore, dietary mercury exposure is a serious challenge for public health.

Acute exposure of mercury has been found to be associated with lung injury. Oxidative stress is considered a hallmark in mercury-induced lung injury (Sener et al., 2007). Oxidative stress represents reactive oxygen species (ROS) overproduction as well as an abrogation of antioxidants (Liu et al., 2017a,b; Yu et al., 2013). Nuclear factor-κB (NF-κB) is one of the cellular sensors which respond to ROS (Topal et al., 2017). NF-κB predominantly presents in the cytosol in an inactive form, which is regulated by inhibitors of NF-κB (IκBs) (Mackenzie et al., 2008). Stressful stimuli, such as hypoxia and ROS, induce NF-κB activity which plays a pivotal role in inflammation, because of its ability of inducing transcription of proinflammatory genes (Park et al., 2016). In addition, ROS is implicated in cell death signaling via regulating both apoptosis and necrosis (Abdel Moneim, 2015). Considering that oxidative stress is involved in mercury toxicity, antioxidants may contribute to the treatment of mercury poisoning.

Luteolin (3′,4′,5,7-hydroxyl-flavone), an active flavonoid compound, is widely distributed in fruits and vegetables, such as chamomile tea, carrots, broccoli, olea europaea L. fruit, etc. Furthermore, recent reports have suggested that certain flavonoids possess abundant pharmacological activities, including antioxidant, anti-inflammatory, and anti-apoptosis, which are also supposed to be the basis of other bioactivities and health benefits (Li et al., 2016; Tan et al., 2018). It is worth of note that the average western diet contains approximately 1 g of flavonoids per day (Kuhnau, 1976). However, the therapeutic potential

Abbreviations: AKT, protein kinase B; Bax, Bcl-2-associated X protein; DMSO, dimethylsulfoxide; GSH, glutathione; H&E, hematoxylin and eosin; $HgCl_2$, mercuric chloride; HO-1, heme oxygenase 1; IL6, Interleukin-6; IκBα, inhibitor of NF-κB alpha; IκBs, inhibitors of nuclear factor-κB; IKK-α, inhibitor of nuclear factor kappa-B kinase α; MDA, malondialdehyde; MPO, myeloperoxidase; NF-κB, Nuclear factor-κB; NQO1, NAD(P)H: quinone oxidoreductase 1; Nrf2, nuclear factor erythroid 2-related factor 2; ROS, reactive oxygen species; SOD, superoxide dismutase; TNF-α, tumor necrosis factor alpha

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of luteolin for mercuric chloride ($HgCl_2$)-induced lung injury and the underlying mechanisms remain incompletely understood.

The nuclear factor erythroid 2-related factor 2 (Nrf2) signaling pathway plays a pivotal role in regulation of adaptive oxidative stress and toxicological response through upregulating an array of antioxidant and detoxifying enzymes (Li et al., 2016). Protein kinase B (AKT) is an important enzyme that regulates cell survival/growth in physiological as well as pathological conditions and also be required for Nrf2 activation (Padiya et al., 2014).

Accordingly, we explored the effects of luteolin in $HgCl_2$-induced lung injury and the molecular mechanisms relevant to the inhibition of NF-κB and activation of AKT/Nrf2 signal pathway.

2. Materials and methods

2.1. Reagents and antibodies

$HgCl_2$ was purchased from Beijing Chemical Plant (Beijing, China). Luteolin (PubChem CID: 5280445) was obtained from Xi'an Weiao Biological Technology Company (Xi'an, China) and its purity was ≥98%. Stock solutions of luteolin were freshly prepared by dissolving it in dimethylsulfoxide (DMSO). The final concentrations of DMSO in the medium were ≤0.1%. Superoxide dismutase (SOD), glutathione (GSH), malondialdehyde (MDA), and myeloperoxidase (MPO) assay kits were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). TUNEL detection kit was obtained from KeyGen Biotech Co. Ltd. (Nanjing, China). Antibodies to Nrf2, heme oxygenase 1 (HO-1), NAD(P)H: quinone oxidoreductase 1 (NQO1), NF-κB, tumor necrosis factor alpha (TNF-α), Interleukin-6 (IL6), IL1β, Bcl-2-associated X protein (Bax), caspase-3, inhibitor of NF-κB alpha (IκBα), and inhibitor of nuclear factor kappa-B kinase (IKK) α were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies against p-IKK-α, p-AKT, AKT, and histone H3 were purchased from Cell Signaling Technology Inc. (MA, USA). The antibody GAPDH was obtained from Hangzhou Goodhere Biotechnology (Hangzhou, China). All secondary antibodies were from ZSGB-BIO (Beijing, China). Nuclear and Cytoplasmic Protein Extraction Kit, BCA protein assay kit, RIPA, and PMSF were obtained from Beyotime Institute of Biotechnology (Shanghai, China).

2.2. Animals and treatments

Forty adult healthy male Kunming mice (20 ± 2 g body weight, 6–8 weeks age), purchased from Experimental Animal Centre of Harbin Medical University (Harbin, China), were acclimated for a week before the start of experimentation. The mice were housed under standard laboratory conditions at a room temperature of 22 ± 2 °C and 55 ± 5% humidity with 12 h interval light/dark cycle. All mice were provided with standard pelleted rodent diet and water *ad libitum*. The animal protocol was conducted according to the Ethical Committee for Animal Experiments of Northeast Agricultural University.

The mice were divided at random into 4 groups: control, luteolin, $HgCl_2$, and $HgCl_2$ + luteolin. The control mice were injected with 0.9% (w/v) physiological saline intraperitoneally for 24 h, followed by intragastrical administration of DMSO. The luteolin mice received luteolin (100 mg/kg) 24 h intragastrically after physiological saline administration. The $HgCl_2$ mice were injected with $HgCl_2$ (5 mg/kg, dissolved in sterile saline) intraperitoneally 24 h, followed by intragastrical administration of DMSO. The $HgCl_2$ + luteolin mice received luteolin 24 h after $HgCl_2$ administration. The dosage of $HgCl_2$ used was based on previously reports (Sener et al., 2007; Yang et al., 2016a). After 24 h, the mice were sacrificed under ether anesthesia.

Lung tissues were rapidly excised and the samples were frozen in liquid nitrogen, and stored at −80 °C until detected.

2.3. Microscopic examination

The lung tissues were fixed in 10% formalin, embedded in paraffin, cut into 5–6 μm sections, and stained with hematoxylin and eosin (H&E). Then, the histological slides were examined by light microscopy (BX-FM, Olympus Corp, Tokyo, Japan).

2.4. TUNEL assay

Apoptotic cell death was assayed on paraffin-embedded sections of lung tissue by the presence of free 3′-hydroxy groups by TUNEL detection kit. The assays were performed as recommended by the manufacturer. Five microscopic fields within the view were randomly selected, and the positive cells were examined and recorded by a fluorescence microscope (Olympus BX41, Nikon, Tokyo, Japan).

2.5. Measurement of bio-markers of oxidative stress

Lung tissues were homogenized in phosphate-buffered saline (PBS) pH 7.4 using an Ultra-Turrax T25 Homogenizer for 10 min. After centrifugation at 2500 r/min for 10 min at 4 °C, SOD activity, and concentration of MDA and GSH were assessed using assay kits following the manufacturer's instruction.

2.6. Measurement of MPO activity

Activity of MPO in the lung tissues was detected with a commercial kit according to the manufacturer's instruction.

2.7. Western blotting

Lung tissues were lysed in RIPA buffer supplement with the 1 mM PMSF. Nuclear and cytosolic proteins were extracted using a Nuclear and Cytoplasmic Protein Extraction Kit according to the manufacturer's instruction. After determination of protein concentration by the BCA method, 30 μg of total protein were loaded on SDS-PAGE and transferred to PVDF membranes. After blocking nonspecific binding sites with 5% nonfat milk in TBST (TBS and 20% Tween 20) for 2 h at room temperature, membranes were incubated overnight at 4 °C with the appropriate concentrations of specific antibodies. Anti-histone and anti-GAPDH were used as loading controls. After washing, the blots were incubated with horseradish peroxidase-conjugated secondary antibodies at 37 °C for 45 min. Three samples were detected and the representative results were shown.

2.8. Statistical analysis

Data analyses were conducted using SPSS 19.0 software (SPSS, Chicago, IL, USA). All data were expressed as mean ± SEM. Statistical analyses were finished by one-way ANOVA and using Tukey's post hoc test to assess significance. Values with $P < 0.05$ were considered as statistically significant.

3. Results

3.1. Effects of luteolin on pulmonary histopathological changes

The HE staining showed that normal pulmonary structures were observed in the control and luteolin groups. In the $HgCl_2$ group, staining revealed widespread increases in alveolar wall thickness, severe hemorrhage in the alveolus, alveolar collapse, and inflammatory cell infiltration. However, when the mice were treated with luteolin, the abovementioned $HgCl_2$-induced pathological changes were attenuated (Fig. 1).