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Quercetin protects mouse liver against nickel-induced DNA methylation and inflammation associated with the Nrf2/HO-1 and p38/STAT1/NF-κB pathway

Chan-Min Liu [a,*], Jie-Qiong Ma [b], Wan-Ru Xie [b], Si-Si Liu [a], Zhao-Jun Feng [a], Gui-Hong Zheng [a], Ai-Min Wang [a]

[a] School of Life Science, Jiangsu Normal University, No. 101, Shanghai Road, Tangshan New Area, 221116, Xuzhou City, Jiangsu Province, China
[b] School of Chemistry and Pharmaceutical Engneering, Sichuan University of Science and Engineering, No. 180, Huixing Road, 643000, Zigong City, Sichuan Province, China

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ABSTRACT

Quercetin (QE), a natural flavonoid, has been reported to have many benefits and medicinal properties. However, its protective effects against nickel (Ni) induced injury in liver have not been clarified. The aim of the present study was to investigate the effects of quercetin on hepatic DNA methylation and inflammation in mice exposed to nickel. ICR mice were exposed to nickel sulfate with or without quercetin co-administration for 20 days. Our results showed that quercetin administration significantly inhibited nickel-induced liver injury, which was indicated by diagnostic indicators. In exploring the underlying mechanisms of quercetin action, we found that quercetin decreased total DNA methyltransferases (DNMTs) activity and DNA methylation level of the NF-E2 related factor 2 (Nrf2) DNA in livers of nickel-treated mice. Quercetin also induced Nrf2 nuclear translocation and heme oxygenase-1 (HO-1) activity. Moreover, quercetin decreased production of pro-inflammatory markers including TNF-α, IL-1β and iNOS. Quercetin significantly inhibited the p38 and signal transducer and activator of transcription 1 (STAT1) activation, which in turn inactivated NF-κB and the inflammatory cytokines in livers of the nickel-treated mice. In conclusion, these results suggested that the inhibition of nickel-induced inflammation by quercetin is associated with its ability to modulate Nrf2/HO-1 and p38/STAT1/NF-κB signaling pathway.

1. Introduction

Nickel (Ni) is considered to be an essential element in microorganisms, plants, and animals, and has been a constituent of enzymes and proteins (Denkhaus and Salnikow, 2002). However, nickel is also considered to be potentially harmful to living creatures, because of its genotoxicity, immunotoxicity, mutagenicity and cancerogenicity (Chervona and Costa, 2012). Nickel is used in modern industry with other metals to form alloys to produce coins, jewelry, and stainless steel as well as for nickel plating and manufacturing Ni-Cd batteries (Konstantin and Zhitkovich, 2008). So exposure to nickel has largely increased in industrial societies due to the environmental pollution by heavy metals at all stages of production, use, and disposal. Exposure to nickel can occur via several different pathways: ingestion, dermal contact, and inhalation (Konstantin and Zhitkovich, 2008). After entering the body, nickel penetrates all organs and accumulates in various tissues and induces the tissue damage. Several researches had demonstrated nickel could cause generation of ROS and increased DNA methylation (Denkhaus and Salnikow, 2002; Konstantin and Zhitkovich, 2008). Our previous study had showed that nickel induced DNA injury in liver and these effects were associated with ROS formation (Liu et al., 2013).

Quercetin (3,5,7,3′,4′-pentahydroxyflavone, QE), one of the most widely distributed flavonoids in fruits, vegetables and red wine, has been reported to possess a wide variety of biological effects, including anti-oxidative, anti-inflammatory, anti-apoptosis, hepatoprotective, renoprotective and neuroprotective effects (Cai et al., 2013; Liu et al., 2010, 2012). Many studies showed that quercetin can protect liver from injury induced by the hazardous metals (Barcelos et al., 2011; Liu et al., 2012; Vicente-Sánchez et al., 2008). Quercetin not only acts as an antioxidant with ability of direct

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; DNMT1, DNA methyltransferase 1; HO-1, heme oxygenase-1; IL-1β, interleukin-1 beta; iNOS, inducible nitric oxide synthase; NF-κB, nuclear factor-κB; Ni, nickel; Nrf2, NF-E2 related factor 2; STAT1, signal transducer and activator of transcription 1; TNF-α, tumor necrosis factor-alpha.

* Corresponding author. School of Life Science, Jiangsu Normal University, No.101, Shanghai Road, Tangshan New Area, 221116, Xuzhou City, Jiangsu Province, China. Tel.: +86 516 83500170; fax: +86 516 83500171.
E-mail address: lcm9009@126.com, lcm9009@jsnu.edu.cn (C.-M. Liu).

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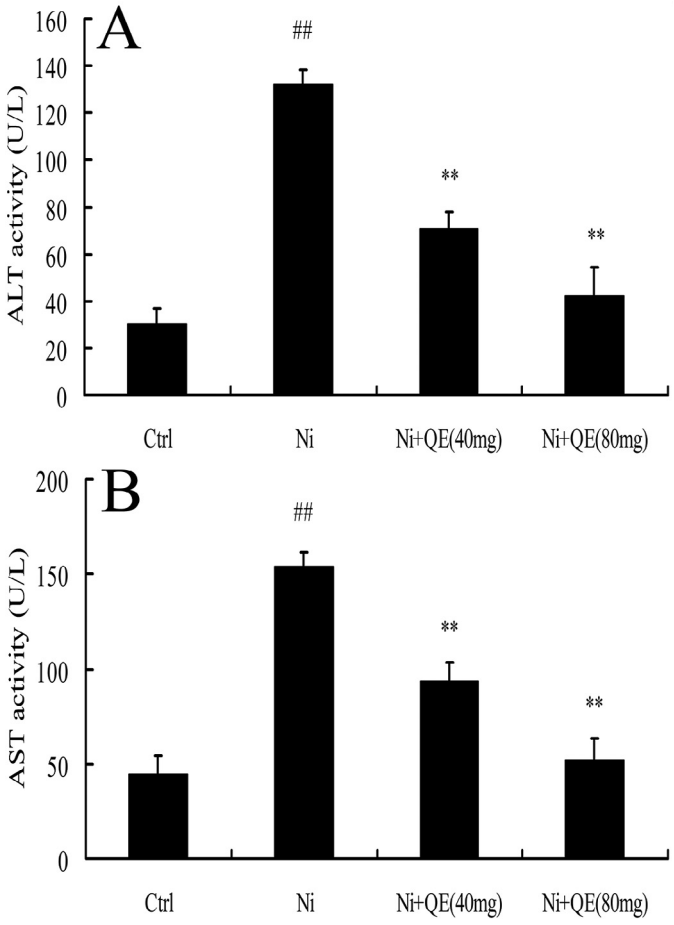

Fig. 1. Effect of quercetin (QE) on nickel-induced changes in hepatic functional markers. (A) ALT activity; (B) AST activity. All values are expressed as mean ± SEM (n = 7). ## $P < 0.01$, compared with the control group; ** $P < 0.01$, vs. the nickel-treated group.

hydrogen-donating properties to quench reactive oxygen species (ROS) via the Michael acceptor function, but also may exert modulatory actions on the endogenous antioxidative defense system by interactions with intracellular signaling cascades (Williams et al., 2004). Heme oxygenase 1 (HO-1), one of antioxidant enzymes, plays a key role in defense mechanisms against oxidative damages. Several evidence had indicated quercetin could induce HO-1 expression and activate nuclear factor erythroid 2 related factor (Nrf2) to bind with the antioxidant response element (ARE) in the HO-1 gene promoter region (Kang et al., 2013; Panchal et al., 2012).

The p38 mitogen-activated protein kinases (MAPKs) signaling pathway plays an important role in inflammation and other physiological processes. Blocking p38 MAPK strongly inhibited production of the major inflammatory cytokines (e.g. tumor necrosis factor-α and interleukin-1) and other proteins (e.g. inducible nitric oxide synthase, cyclooxygenase-2) (Liu et al., 2012; Saklatvala, 2004). The activated p38 MAPK kinase is known to phosphorylate STAT1 (signal transducer and activator of transcription 1) at Ser-727 and induces inflammatory signal transcription factor expression (Lim et al., 2013; Rhee et al., 2003). Previous studies reported that nickel-induced inflammation was associated with the STAT1/NF-κB pathway (Antonios et al., 2010; Nemec and Barchowsky, 2009). In the present study, we aimed to determine whether quercetin can protect mouse liver against nickel-induced DNA damage and inflammation associated with modulating the Nrf2/HO-1 and p38/STAT1/NF-κB pathway.

2. Materials and methods

2.1. Chemicals and reagents

Quercetin (>98% purity) and nickel sulfate ($NiSO_4$) were obtained from Sigma Chemical Co. (St. Louis, MO, USA); phospho-STAT1(Ser-727) antibody, STAT1 antibody, phospho-p38 antibody, p38 antibody, NF-κB p65 antibody, TNF-α antibody, IL-1β antibody and iNOS antibody from Santa Cruz Biotechnology (Santa Cruz, CA, USA) or Cell Signaling Technology (Beverly, MA, USA); phospho-STAT1(Ser-727); alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in serum was determined using the assay kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China); protein concentration were measured using the BCA assay kit from Pierce Biotechnology, Inc. (Rockford, IL, USA). All other reagents unless indicated were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Animals and treatment

Male ICR mice (4 weeks old weighing approximately 18 g) were purchased from the Branch of National Breeder Center of Rodents (Beijing). The mice were maintained under constant conditions (23 ± 1 °C and 60% humidity) with free access to rodent food and tap water under 12 hours light/dark schedule (lights on from 08:30 to 20:30 hour).

After acclimatization to the laboratory conditions, the mice were randomly divided into four groups of ten mice each. Group I: The mice served as control (treated intraperitoneally with physiological saline for 20 days); Group II: The mice received intraperitoneally nickel sulfate (20 mg/kg/body weight, daily) following a daily oral gavage administration of physiological saline; Groups III and IV: The mice received intraperitoneally nickel sulfate (20 mg/kg/body weight, daily) following a daily oral gavage administration of quercetin (40 and 80 mg/kg body weight, dissolved in physiological saline); the choice of nickel sulfate dose is based on previous findings and indicates the level of toxic intake of nickel in occupational exposure and some emergency (Liu et al., 2013). The choice of quercetin dose is based on previous findings, which showed that quercetin has protective effects on tissue damage (Ji et al., 2014).

The experiment lasted for 20 days. At the end of treatment, mice were sacrificed and blood samples were drawn by cardiac puncture with heparinized tubes. Liver tissues were quickly collected, placed in ice-cold 0.9% NaCl solution, perfused with the physiological saline solution to remove blood cells, blotted on filter paper, and stored at –70 °C for later use.

This research was conducted in accordance with Chinese laws and NIH publications on the use and care of laboratory animals. Relevant university committees for animal experiments approved these experiments.

2.3. Measurement of serum aminotransferase activities

The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum were estimated spectrophotometrically using commercial diagnostic kits (Jiancheng Institute of Biotechnology, Nanjing, China) (Liu et al., 2013).

2.4. Histological evaluations

The histological changes of liver were evaluated as described by ours (Liu et al., 2013). The extent of hepatic damage was evaluated on H&E slides. The histological changes were scored according to the following criteria: 0, absent; 1, mild; 2, moderate; and 3, severe (Kim et al., 2011).

2.5. Measurement of total DNA methyltransferases (DNMTs) activity

The total DNMT activity was measured using EpiQuik TM DNA methyltransferase activity assay kit (Epigentek Group Inc. USA). Absorbance was determined using a microplate spectrophotometer at 450 nm, and DNMT activity (OD/h/mg) was calculated according to the manufacturer's instructions (Huang et al., 2013a).

2.6. Methylation-specific PCR

Methylation of Nrf2 DNA was measured as described previously (Kang et al., 2014). Briefly, MS-PCR was performed using an Epitect MSP kit (Qiagen, Hilden, Germany). PCR products were separated on 6% nondenaturing polyacrylamide gels, stained with ethidium bromide, and visualized under UV light. The primer sets were as follows: for unmethylated forward 5′-GGAGGTGTAGTTTTTATATTAATGT-3′ and unmethylated reverse, 5′-ACCAACTAAAATCCCAACAAACA-3′; for methylated forward, 5′-AGGGAGGCGTAGTTTTTATATTAAC-3′ and methylated reverse, 5′-AACTAAAATCCCAA CAAACGAA-3′.

2.7. Western blot analyses

Tissues were homogenized in ice-cold lysis buffer (TBS, 1% NP-40, 0.25% sodium deoxycholate, 1 mM EDTA, 10 mg/ml Aprotenin, 10 mg/ml Leupeptin, 1 mM PMSF, 10 mM β-glycerophosphate, 1 mM Na_3VO_4, 1 mM NaF). Homogenates were centrifuged at 10,000 *g* for 10 min at 4 °C. The supernatants were collected and centrifuged again and the final supernatants were collected. Nuclear and cytoplasmic extracts