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Naringin protects against perfluorooctane sulfonate-induced liver injury by modulating NRF2 and NF-κB in mice

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ABSTRACT

Perfluorooctane sulfonate (PFOS), a persistent organic pollutant, has been demonstrated to cause multiple toxicities. In this study, we explored the role of naringin (Nar) in alleviating PFOS-caused mouse liver injury and its potential mechanisms. Male mice were intragastrically administered PFOS (10 mg/kg/day) alone or with Nar (100 mg/kg/day) for 3 weeks. Nar supplementation led to resumption of elevated serum hepatic enzyme activities and increased relative liver weight in PFOS-challenged mice. Moreover, Nar treatment increased hepatic expression of transcription factor NRF2 protein and its regulated antioxidative enzyme genes heme oxygenase-1, superoxide dismutase and catalase, with an inhibition of malondialdehyde and hydrogen peroxide production. Furthermore, simultaneous administration of Nar suppressed PFOS-induced elevation in NF-κB activity and generation of inflammatory cytokines TNF-α and IL-6 in the liver. In addition, Nar enhanced anti-apoptotic Bcl-2 expression, decreased pro-apoptotic Bax expression and inhibited caspase-3 activation in liver tissue in mice exposed to PFOS. Our results indicate that Nar protects against PFOS-induced hepatotoxicity in mice via modulating oxidative, inflammatory and apoptotic pathways.

1. Introduction

Exposure to environmental contaminants is an important source of health risk. Perfluorooctane sulfonate (PFOS), a member of fluorinated organic compounds, has raised considerable public health concerns. As an ultimate degradation and metabolism product of many commercially used perfluorinated chemicals (PFCs), PFOS is regarded as a persistent environmental pollutant that has been extensively found in worldwide populations due to its bioaccumulative ability and long human half-life of serum elimination [1]. Especially, occupationally exposed people have much higher serum PFOS levels than general population [2]. An investigation in China from 2008 to 2012 showed that the median serum PFOS concentration reached 5544 ng/mL (range, 416 to 118,000 ng/mL) in workers from sulfonation department of a fluorochemical plant [3]. The accumulated levels of PFCs in the tissue may be associated with adverse health outcomes. Epidemiological evidence has demonstrated that PFOS exposure is positively related to increased carotid intima-media thickness and elevated serum lipid, total bilirubin and alanine transaminase levels [4–7]. Animal studies have also revealed that PFOS exerts toxic action on development and immunity [8]. As the predominant organ for PFOS accumulation, the liver is primarily damaged by PFOS-induced toxicity [9,10]. However, the toxic mechanisms of PFOS-induced hepatic damage has not been elucidated fully.

Nutritional interventions may be an effective approach to attenuate the toxicity of environmental pollutants [11]. Citrus fruits represent a main source of compounds with health promoting activities [12]. Naringin (Nar, 4′,5,7-trihydroxyflavonone-7-rhamnoglucoside), a naturally occurring flavonoid glycoside isolated from citrus fruits, displays multiple pharmacological actions including antioxidant, antibacterial, anti-apoptotic and anti-inflammatory properties [13]. Most of the biological actions of Nar are associated with expression of cell survival proteins and modulation of signaling pathways [14,15]. Accumulating evidence indicates that Nar exerts therapeutic effects in preclinical models such as cardiovascular disease, diabetes mellitus, metabolic syndrome, neurodegeneration, hepatic injury, osteoporosis and cancer, suggesting a potential curative application in human disorders [14].

Studies have reported that Nar plays a protective role in hepatotoxicity caused by carbon tetrachloride, sodium arsenite, nickel and

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thioacetamide [16–19]. In this study, the protection by Nar from PFOS-caused hepatic injury was evaluated to gain more insight into the healthy contribution of Nar.

2. Materials and methods

2.1. Animals and treatments

Male mice (20–22 g) were procured from Animal Science Department of Nanchang University and were maintained in standard housing conditions of temperature (22 °C) and light (12-h light/12-h dark). All procedures involving animals were performed under the Guide to Care and Use of Experimental Animals, and were permitted by Animal Ethics Committee (no. 20130916). Mice were randomly assigned to receive 10 mg/kg PFOS (≥98%, Sigma-Aldrich), 100 mg/kg Nar (≥95%, Sigma-Aldrich), or their combination once per day via the intragastric route. Control mice were given the same volume of vehicle. After treatment for 3 weeks, fasting venous blood was collected from mice under anesthesia by retro-orbital plexuspuncture, and liver samples were stored in −80 °C freezer and fixed in 10% buffered formalin.

2.2. Serum biochemistry

Serum activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) were assayed by using enzyme kits supplied by Jiancheng Bioengineering Institute (Nanjing, China).

2.3. Histopathology

The fixed liver tissues were dehydrated through a graded series of alcohols and infiltrated with paraffin. The embedded tissue was cut into 5 μm sections, followed by routine hematoxylin-eosin staining for histological estimation.

2.4. Oxidative status analysis

The production of malondialdehyde (MDA), generation of hydrogen peroxide (H_2O_2), content of glutathione (GSH) and activity of superoxide dismutase (SOD) were detected in homogenized liver to assess oxidative stress and antioxidant status by utilizing the test kits (Jiancheng Bioengineering Institute, China).

2.5. Protein extraction and Western blotting

Liver homogenization was carried out in RIPA buffer with 1 mM phenylmethanesulfonyl fluoride and the lysate was cleared by centrifugation. The supernatant was harvested for immunoblotting analysis. After fractionation by 12% SDS-PAGE, proteins were electrotransferred to nitrocellulose, and analyzed by immunoblotting with antibodies to NRF2, Bax, Bcl-2, p53, NF-κB p65, phospho-p65 (p-p65) (Cell Signaling Technology, USA), or GAPDH (OriGene Technologies, USA). Then, the blots were rinsed with TBST buffer and incubated at room temperature for 2 h with anti-rabbit secondary antibody conjugated to horseradish peroxidase (OriGene Technologies, USA). Bands were developed with ECL system (Thermo Fisher Scientific, USA).

2.6. RNA extraction and quantitative PCR

The total hepatic RNA was isolated from tissue homogenized in Trizol reagent, and then was converted into cDNA with a reverse transcription kit (TaKaRa Bio, China). PCR amplification was carried out using SYBR Green on an ABI Prism 7500 Real-time PCR system (Applied Biosystems, USA). The mRNA expression for *HO-1*, *SOD* and *CAT* was normalized using *GAPDH* as a housekeeping gene (Table 1). Relative quantification of target genes was conducted by the $2^{-\Delta\Delta CT}$ method.

Table 1
Primer sequences for real-time PCR assay.

Gene	Sequences (5′-3′)	Product length (bp)
HO-1	F: CCTCACAGATGGCGTCACTT	92
	R: GCTGATCTGGGGTTTCCCTC	92
SOD	F: ATCCACTTCGAGCAGAAGGC	96
	R: CTGATGGACGTGGAACCCAT	96
CAT	F: TTTTGCCTACCCGGACACTC	154
	R: GGGGTAATAGTTGGGGGCAC	154
GAPDH	F: GGCAAATTCAACGGCACAGT	84
	R: GTCTCGCTCCTGGAAGATGG	84

2.7. Measurement of caspase-3 activity

Enzymatic activity of caspase-3 in hepatic tissue homogenate was measured by colorimetric method according to manufacturer's protocol (KeyGen Biotech, China).

2.8. Determination of proinflammatory cytokines

Concentrations of inflammatory mediators interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) in mouse liver were analyzed with ELISA kits (Cloud-Clone Corp, USA).

2.9. Statistical analysis

Data were analyzed statistically by one-way analysis of variance with Graph Pad Prism 5.0 (GraphPad, USA), and represented as mean value ± standard deviation (SD) of 4 mice. Differences were regarded as significant when P-value was < 0.05.

3. Results

3.1. Nar reduced serum hepatic enzyme activities in PFOS-exposed mice

After 21 days of intragastric PFOS exposure, mice developed serious liver injury which was evidenced by elevated serum activities of AST, ALT and LDH (Fig. 1A–C), as well as increased relative liver weight (Fig. 1D) ($P < 0.05$). However, the elevated hepatic enzyme levels were restored and the relative liver weight was reduced by simultaneous supplementation of Nar in PFOS-treated mice ($P < 0.05$). No remarkable differences in serum levels of these biochemical markers of liver function were found between control group and Nar treatment group ($P > 0.05$).

3.2. Nar ameliorated PFOS-caused hepatic histopathologic alterations

As indicated in Fig. 2, PFOS administration led to distinct histopathologic changes in mouse liver, which were characterized by inflammatory cell infiltration, architectural disorganization, marked edema, cytoplasmic vacuolation and focal necrosis. Nevertheless, PFOS-induced pathologic alterations were significantly attenuated by Nar supplementation. The mice only dosed with Nar exhibited a normal liver architecture as the untreated animals.

3.3. Nar relieved PFOS-induced hepatic oxidative injury

As presented in Fig. 3, PFOS challenge stimulated the production of MDA and H_2O_2, suppressed the activity of SOD and reduced the content of GSH in liver homogenates, in comparison with control group ($P < 0.05$). Nonetheless, combined treatment with Nar prevented the elevation of MDA and H_2O_2 levels and inhibited the decline in SOD activity and GSH content ($P < 0.05$). When compared to untreated