Chemico-Biological Interactions 286 (2018) 26–33

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

Protective effect of rutin against carbon tetrachloride-induced oxidative stress, inflammation and apoptosis in mouse kidney associated with the ceramide, MAPKs, p53 and calpain activities

Jie-Qiong Ma[a,*], Chan-Min Liu[b], Wei Yang[b]

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

ARTICLE INFO

Keywords:
Rutin
CCl_4
Kidney
Oxidative stress
Inflammation
Apoptosis

ABSTRACT

Rutin, a natural flavonoid, possess beneficial health effects. However, its renoprotective effect against carbon tetrachloride (CCl_4) induced injury and the underlying mechanism is not clarified. The current study aims is to identify the therapeutic effects of rutin on oxidative stress, inflammation and apoptosis in mouse kidney exposed to CCl_4. ICR mice received CCl_4 with or without rutin co-administration for one week. Compared with the control group, mice receiving CCl_4 alone showed kidney injury as evidenced by elevation in serum biochemical markers, inflammation, caspase-3 activity and apoptosis in kidney, while rutin administration significantly attenuated these pathophysiological changes. Exploration of the underlying mechanisms of its action demonstrated that rutin reduced the ROS, calpain and ceramide levels in mouse kidneys. Rutin significantly decreased the p53, TNF-α, IL-1β activities and mitogen-activated protein kinase (MAPK) phosphorylation in the kidneys. In addition, rutin increased the levels of Bcl-2 protein and reduced levels protein of Bax. Rutin also inhibited the release of cytochrome C from mitochondria in kidneys of the CCl_4-treated mice. Taken together, rutin ameliorates CCl_4-induced oxidative stress, inflammation and apoptosis through regulating the ceramide, MAPK, p53 and calpain activities and thereby suppressing apoptosis by the mitochondrial pathway.

1. Introduction

Ceramide, a central core of a signaling network of sphingolipid metabolism, is involved in a variety of important functional responses including apoptosis, inflammation, and stress responses [1–3]. Ceramide is synthesized upon stimuli. Overexpressed ceramides may directly participate in the generation of free radicals in mitochondria and provoke mitochondrial dysfunction and apoptosis [3,4]. ceramide could modulate the immune response directly by signaling pathways, or indirectly by cytokines produced by other cells [5]. Ceramide could affect many targets in apoptotic signaling pathways include mitochondria, lysosomal cathepsin D, mitogen-activated protein kinases (MAPKs), Ca^{2+} homeostasis, protein phosphatases, Bcl-2 family members and protein kinase C [6]. Carbon tetrachloride (CCl_4) is a haloalkane used in lots of industrial and chemical applications as a solvent for oils, fats, lacquers, varnishes and resins, as well as a primary material in the production of organic compounds [7]. CCl_4 is also a well-known toxin, which causes tissue damage in human and animals [7–9]. Many studies have demonstrated that CCl_4 could increase ceramide content in livers and kidneys [8,9].

Rutin (3,3′,4′,5,7-pentahydroxyflavone-3-rhamnoglucoside) is consumed in the daily diet, such as buckwheat, tomato, orange, lemon, apple, and tea [10–13]. Rutin is a citrus flavonoid glycoside, abundantly found in buckwheat and other plants [12,13].

Rutin had previously been reported to show anti-oxidative, anti-glycation, anti-inflammatory, anti-diabetic, gastroprotective, hepatoprotective and renoprotective effects [10–13]. Rutin exerted the protective effects on several targets include pancreatic islets, liver, kidney, brain, against hyperglycemia-induced diseases in model animals [10,11]. Rutin could alleviate doxorubicin-induced cardiotoxicity by inhibiting autophagy and apoptosis in mice [13]. Rutin could modulate microglial/macrophage activation to a CD150/CD206 M2 phenotype [14]. Rutin is considered as an effective skin fibroblasts protector against UV-induced changes in structure and functions of phospholipid membrane [15]. Rutin could inhibit oxidative stress and inflammation in model animals via regulating MAPK pathway [11,16]. Rutin had

Abbreviations: ROS, reactive oxygen species; JNK, the c-Jun N-terminal kinases; ERK, the extracellular-receptor kinases; MAPKs, mitogen-activated protein kinases; MDA, malondialdehyde; ICR, Institute of Cancer Research

* Corresponding author.
E-mail address: a1032042419@126.com (J.-Q. Ma).

https://doi.org/10.1016/j.cbi.2018.03.003
Received 10 June 2017; Received in revised form 24 February 2018; Accepted 5 March 2018
Available online 06 March 2018
0009-2797/

Table 1
Effect of rutin on CCl_4-induced changes in renal functional markers.

Group	Urea (mg/dl)	Uric acid(mg/dl)	Creatinine(mg/dl)
Control	37.21 ± 3.84	1.26 ± 0.21	1.12 ± 0.23
CCl_4	65.26 ± 4.91##	2.51 ± 0.19##	2.53 ± 0.15##
CCl_4 + Rutin(75 mg)	53.11 ± 4.15**	2.02 ± 0.17**	2.21 ± 0.12**
CCl_4 + Rutin (150 mg)	49.05 ± 3.58**	1.51 ± 0.23**	1.57 ± 0.19**

Data are reported as Mean ± SE. Statistical significance was analyzed by generalized linear model followed by post hoc Tukey's multi-comparison test. ##$P < 0.01$, compared with the control group; **$P < 0.01$, vs. CCl_4-treated group.

exhibited its gastroprotective effect by inhibiting oxidative stress and preventing neutrophil infiltration in indomethacin-induced gastric damage [17,18]. Moreover, researcher showed that rutin had stronger protective effects than quercetin effects against nitrosative stress and hepatocellular damage [19]. For these reasons, we hypothesized that rutin might play an important role in protecting CCl_4-induced kidney injury. Herein, we examined protective effects of rutin on CCl_4-induced kidney injury and disclosed the mechanisms focusing on these effects in inhibiting oxidative stress, inflammation and apoptosis by modulating the ceramide, MAPK and calpain pathway.

2. Materials and methods

2.1. Chemicals and reagents

Rutin (> 98% purity) was purchased from Sigma (St. Louis, MO, USA); The antibodies against phospho-p38, p38, phospho-JNK1/2, JNK1/2, phospho-ERK1/2, ERK1/2, TNF-α, IL-1β, p53, calpain, Bcl-2, Bax, cleaved caspase-3 and cytochrome *c* from Santa Cruz Biotechnology (CA, USA).

2.2. Animals and treatment

The entire experimental procedures were conducted in accordance with the Chinese legislation and NIH publication on the use and care of laboratory animals. This study was approved by the respective university committees for animal experiments.

Male ICR (Institute of Cancer Research) mice (20–25 g) were randomly assigned to four groups (10 mice/group). Kidney damage was induced by intraperitoneal (i.p.) injection of 2 ml/kg of CCl_4 in peanut oil (1:1, v/v) twice weekly for up to one week. Mice in Group 1 were given twice weekly injections of peanut oil (vehicle control); Mice in Group 2 were injected with CCl_4; mice in Group 3 and Group 4 were injected with CCl_4, as in group 2, and daily given rutin at two doses 75 and 150 mg/(kg day) by oral gavage, respectively. The choice of rutin dose is based on previous reports [20,21].

At the end of treatment, mice were anesthetized to collect the blood sample and then sacrificed to obtain the kidneys.

2.3. Assay of serum urea, uric acid and creatinine levels

The commercial kits of Jiancheng Institute of Biotechnology (Nanjing, China) were used in the assays of the urea, uric acid and creatinine in serum [22].

2.4. Deoxyribonucleotidyl transferase (TdT)-mediated dUTP-uorescein isothiocyanate (FITC) nick-end labeling (TUNEL) assay

Apoptosis was assayed by TUNNEL staining using commercial diagnostic kits (BD Biosciences Clontech, Palo Alto, CA, USA) [23].

2.5. Assay of reactive oxygen species (ROS) level

ROS was determined as described in others and our previous reports [23–25].

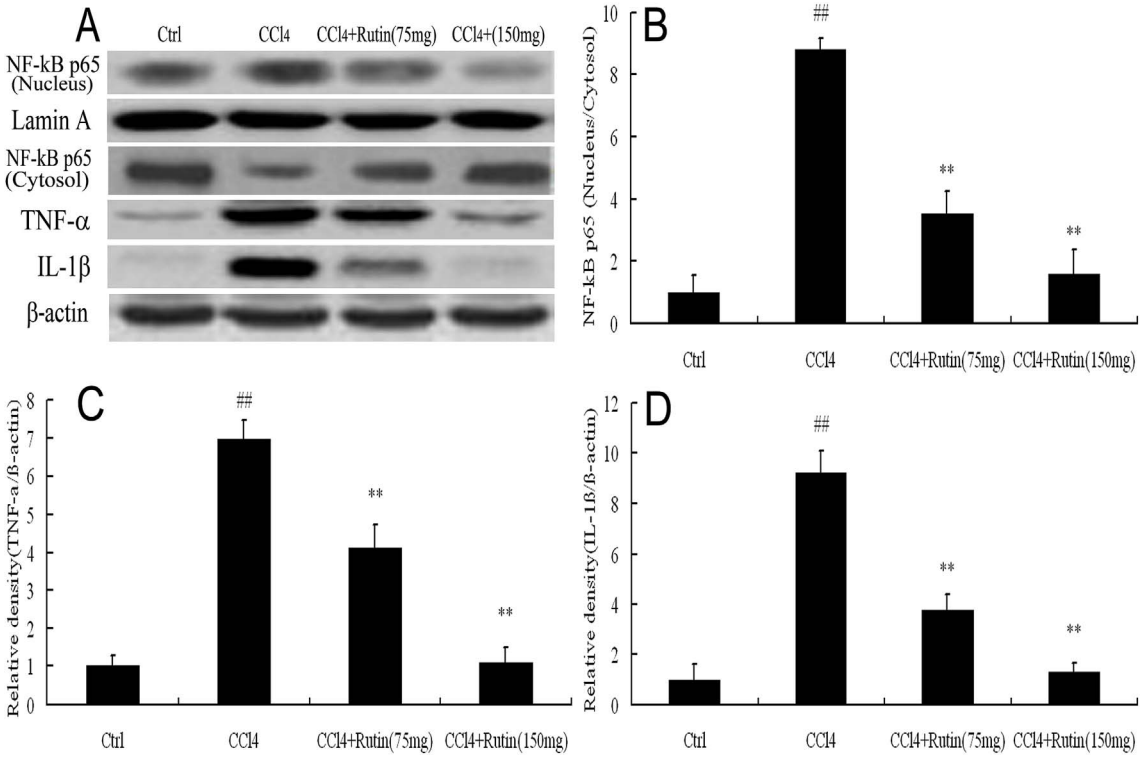

Fig. 1. Western blot analysis of inflammatory cytokine levels in the kidneys of mice. (A) Protein expression levels of inflammatory cytokine; (B) Relative density analysis of NF-κB p65; (C) Relative density analysis of TNF-α; (D) Relative density analysis of IL-1β. β-Actin was probed as an internal control in relative density analysis. The vehicle control is set as 1.0. Each value is expressed as mean ± S.E.M. (n = 7). All values are expressed as mean ± SD. (n = 7). ##$P < 0.01$, compared with the control group; **P < 0.01, vs. CCl_4-treated group.