Available online at www.sciencedirect.com

ScienceDirect

Journal of Nutritional Biochemistry 25 (2014) 1045–1057

Journal of Nutritional Biochemistry

DJ-1 plays an important role in caffeic acid-mediated protection of the gastrointestinal mucosa against ketoprofen-induced oxidative damage

Yu-Ting Cheng[a], Cheng-Ying Ho[a], Jhih-Jia Jhang[a], Chi-Cheng Lu[a], Gow-Chin Yen[a,b,*]

[a]Department of Food Science and Biotechnology, National Chung Hsing University, 250 Kuokuang Road, Taichung 40227, Taiwan
[b]Agricultural Biotechnology Center, National Chung Hsing University, 250 Kuokuang Rd., Taichung 40227, Taiwan

Received 30 December 2013; received in revised form 30 April 2014; accepted 8 May 2014

Abstract

Ketoprofen is widely used to alleviate pain and inflammation in clinical medicine; however, this drug may cause oxidative stress and lead to gastrointestinal (GI) ulcers. We previously reported that nuclear factor erythroid 2-related factor 2 (Nrf2) plays a crucial role in protecting cells against reactive oxygen species, and it facilitates the prevention of ketoprofen-induced GI mucosal ulcers. Recent reports suggested that Nrf2 becomes unstable in the absence of DJ-1/PARK7, attenuating the activity of Nrf2-regulated downstream antioxidant enzymes. Thus, increasing Nrf2 translocation by DJ-1 may represent a novel means for GI protection. In vitro, caffeic acid increases the nuclear/cytosolic Nrf2 ratio and the mRNA expression of the downstream antioxidant enzymes, γ-glutamyl cysteine synthetase, glutathione peroxidase, glutathione reductase, and heme oxygenase-1, by activating the JNK/p38 pathway in Int-407 cells. Moreover, knockdown of DJ-1 also reversed caffeic acid-induced nuclear Nrf2 protein expression in a JNK/p38-dependent manner. Our results also indicated that treatment of Sprague–Dawley rats with caffeic acid prior to the administration of ketoprofen inhibited oxidative damage and reversed the inhibitory effects of ketoprofen on the antioxidant system and DJ-1 protein expression in the GI mucosa. Our observations suggest that DJ-1 plays an important role in caffeic acid-mediated protection against ketoprofen-induced oxidative damage in the GI mucosa.

Keywords: Caffeic acid; Gastrointestinal ulcer; HO-1; Ketoprofen; Nrf2; DJ-1; NSAIDs

1. Introduction

Peptic ulcer caused by mucosal damage is a common digestive disease in modern medicine, and it affects approximately 8–10% of the global population [1]. The gastrointestinal (GI) mucosa serves as the first protective barrier for digested foods and xenobiotics. Therefore, the GI mucosa is easily damaged by toxic agents, such as alcohol, tobacco, and drugs [2]. Non-steroidal anti-inflammatory drugs (NSAIDs), such as ketoprofen, are widely used in clinical medicine to alleviate the pain and swelling associated with rheumatoid arthritis and other inflammatory disorders; however, the use of these drugs may cause peptic ulcers. Serious ulceration may lead to GI bleeding or even perforation. Several reports have indicated that digestive diseases, such as ulcers, are associated with lipid peroxidation and oxidative damage in the mucosa [3–5]. Therefore, reducing oxidative stress may be an effective therapeutic strategy for preventing and treating ketoprofen-induced GI mucosal ulcers.

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a central transcription factor that binds to an antioxidant response element (ARE) in the promoter region of genes that encode several detoxifying and antioxidant enzymes and related stress-responsive proteins. Under normal conditions, Nrf2 is bound by Kelch-like ECH-associated protein 1 (Keap1) in the cytosol and remains inactive. The cytoplasmic protein, Keap1, sequesters stimuli that modify thiol groups, including electrophilic agents, oxidants, antioxidants, and chemopreventive agents, and liberation of Nrf2 from Keap1 allows it to translocate into the nucleus and bind to the ARE [6]. Nrf2 induces rescue pathways that protect cells against oxidative damage, abnormal inflammation, abnormal immune responses, apoptosis and carcinogenesis [6,7]. In our previous study, we reported that sulforaphane effectively prevents indomethacin-induced injury in human Int-407 cells by up-regulating heme oxygenase-1 (HO-1) expression via the Nrf2 pathway [8]. Recent reports indicate that cytosolic Nrf2 becomes unstable in the absence of DJ-1 (also known as PARK7), attenuating the response of Nrf2-regulated downstream antioxidant enzymes [9,10]. Pathogenic mutations in DJ-1 have been

Abbreviations: ARE, antioxidant response element; CAT, catalase; COX, cyclooxygenase; GI, gastrointestinal; GSH, glutathione; GSSG, glutathione disulfide; GPx, glutathione peroxidase; GRd, glutathione reductase; HO-1, heme oxygenase-1; Int-407 cells, intestinal-407 cells; Keap-1, Kelch-like ECH-associated protein 1; LPS, lipopolysaccharide; LDH, lactate dehydrogenase; MAPK, mitogen-activated protein kinases; MDA, malondialdehyde; MPO, myeloperoxidase; NSAIDs, Non-steroidal anti-inflammatory drugs; PG, prostaglandin; NQO1, NAD(P)H:(quinone-acceptor) oxidoreductase 1; Nrf2, Nuclear factor erythroid 2-related factor 2; ROS, reactive oxygen species; RPG cells, rat primary gastric mucosal cells; SD rats, Sprague–Dawley rats; SOD, superoxide dismutase; TBA, thiobarbituric acid; TSH, total sulfhydryl groups.

* Corresponding author. Tel.: +886 4 2287 9755; fax: +886 4 2285 4378.
E-mail address: gcyen@nchu.edu.tw (G.-C. Yen).

http://dx.doi.org/10.1016/j.jnutbio.2014.05.007
0955-2863/

linked with a familial form of early onset Parkinson's disease and neurodegenerative disorders [11]. Taira et al. [12] reported that up-regulation of DJ-1 prevents neuroblastoma cell death due to oxidative stress in Parkinson's disease. However, the role of phytochemicals and DJ-1 in GI protection remains unclear.

In contrast, many reports have suggested that nuclear translocation of Nrf2 requires the activation of mitogen-activated protein kinases (MAPKs), such as the JNK, ERK1/2, and p38 pathways [6–8]. Ren et al. [13] reported that DJ-1 regulates autophagy in a JNK-dependent manner. He et al. [14] suggested that DJ-1 promotes cell migration via the ERK/uPA cascade in pancreatic cancer cells. In our previous study, we found that Nrf2 played a crucial role in the protection of cells against ROS and ketoprofen-induced GI mucosal ulcers [15]. An effort to screen a series of compounds in our laboratory (data not shown) revealed that phytochemicals (theaflavin, caffeic acid, ferulic acid, malvidin, cyanidin, apigenin, lycopene, and β-carotene) exhibited the potential to induce glutathione peroxidase (GPx) activity in Int-407 cells, which might play an important role in GI protection. However, the role of phytochemicals in modulating DJ-1/Nrf2 via MAPKs is still unclear. In this study, we propose that increasing DJ-1/Nrf2 expression using exogenous antioxidant compounds represents a potential therapeutic approach to protect patients with oxidative stress-associated disorders from developing GI ulcers.

2. Materials and methods

2.1. Materials

Caffeic acid, ferulic acid, lycopene, β-carotene, Zn (II) protoporphyrin IX (ZnPP IX), S(+)-ketoprofen and 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased form Sigma Chemical Co. (St. Louis, MO, USA). Apigenin, cyanidin, and malvidin were purchased from Extrasynthese (Lyon, France). 2′-7′-dichloro-fluorescein diacetate (DCFH-DA) was purchased from Molecular Probes (Eugene, OR, USA). Eagle's basal medium, bovine serum, trypsin-EDTA, L-glutamine, and penicillin-streptomycin-neomycin antibiotic mixture were purchased from Gibco (Grand Island, NY, USA). The Trizol RNA isolation kit was obtained from Life Technologies (Rockville, MD, USA). Primers for RT-PCR, dNTPs, reverse transcriptase, and Taq polymerase were obtained from Gibco BRL (Cergy Pontoise, France). The inhibitors of MAPKs, SB203580, and SP600125 were obtained from Biosource (Camarillo, CA, USA). Anti-ERK, anti-phospho-ERK, anti-p38, anti-phospho-p38, anti-JNK, anti-phospho-JNK, anti-lamin B1, anti-β-actin, anti-Nrf2, and anti-DJ-1 antibodies were obtained from Cell Signaling Technology (Beverly, MA, USA). The anti-HO-1 antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The anti-mouse and anti-rabbit antibodies were purchased from Bethyl Laboratories (Montgomery, TX, USA). All other chemicals and solvents used were of the highest purity available.

2.2. Cell culture

The human intestinal epithelial cell line, Int-407 (BCRC 60022), was purchased from the Bioresource Collection and Research Center (BCRC) of the Food Industry Research and Development Institute (Hsinchu, Taiwan). Rat primary gastric mucosal (RPG) cells were isolated from male Sprague–Dawley (SD) rats (100–125 g) as previously reported methods [15–17]. The cells were cultured in BME/EBSS supplemented with 10% BS and 1% PSN and maintained at 37°C in a humidified atmosphere of 95% air and 5% CO_2. The culture medium was renewed each day. The cells were detached with 0.1% trypsin and 10 mM EDTA in phosphate-buffered saline (PBS), and the cells were split weekly.

2.3. Transfection of small interfering RNA against Nrf2 (si-Nrf2) and DJ-1 (si-DJ-1)

Plasmids containing si-Nrf2 or si-DJ-1, and sureFECT transfection reagent were purchased from Qiagen (Valencia, CA, USA). The si-Nrf2 and DJ-1 solutions were diluted with DEPC water. The transfection reagent was mixed with si-Nrf2 or si-DJ-1, incubated for 20 min, and then added to the culture medium for 24 h.

2.4. Cell survival assay

Cell viability was determined using MTT assays. Briefly, 500 μL of MTT solution (5 mg/mL in PBS) was added to each well, and the cells were cultured at 37°C in a humidified incubator with an atmosphere of 5% CO_2 for 4 h. The medium was aspirated to facilitate formazan formation, which was then solubilized by the addition of 500 μL of DMSO. The optical density was measured at 570nm with a FLUOstar Galaxy fluorescence plate reader (BMG Lab Technologies, Offenburg, Germany).

2.5. Immunofluorescence analysis

The cells were fixed in 2% paraformaldehyde for 10min at room temperature, permeabilized with 0.1% Triton X-100 in 0.01 MPBS (pH7.4) containing 0.2% BSA, air-dried, and rehydrated in PBS. Then, the cells were incubated with a rabbit polyclonal antibody against Nrf2 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), which was diluted 1:500 in PBS containing 3% normal goat serum, for 1.5 h at room temperature. For negative controls, the primary antibody was omitted. After two 10 min washes in PBS, an anti-rabbit IgG PE-conjugated secondary antibody (Jackson Immunoresearch Laboratories, West Grove, PA, USA), which was diluted 1:500 in PBS, was added for 1h at room temperature. The cells were then washed in PBS and mounted in Vectashield mounting medium containing 4′,6-diamidino-2-phenylindole (DAPI) to counterstain the DNA. The cells were visualized using an Olympus fluorescence microscope (Osaka, Japan). Images were acquired using a digital video camera (Olympus, Osaka, Japan) and ImagePro (Media Cybernetics, Bethesda, MD, USA) software.

2.6. Experimental animals and procedures

Male SD rats (175–200 g) were purchased from BioLASCO Co. (Taipei, Taiwan). The animals were housed individually in stainless steel cages and maintained in an air-conditioned room at 22–24°C with 65–70% relative humidity on a 12/12 h light/dark cycle, and they were fed a normal laboratory diet for 1week. All experimental procedures involving animals were performed in accordance with the National Institutes of Health (NIH) guidelines. These experiments were approved by the Institutional Animal Care and Use Committee (IACUC Approval 100–21) of the National Chung Hsing University (Taichung, Taiwan). The animals were orally administrated according to previously reported methods with some modifications [4,18]. Briefly, the animals received caffeic acid (60 or 120 mg/kg per day) orally for three weeks. The control group received vehicle (ddH_2O) only. On day 21, ketoprofen (50 mg/kg per day) was orally administered to all animals, and the rats were sacrificed with CO_2 after 24 h.

2.7. Preparation of mucosa homogenate

Extraction of the mucosa was performed according to a previously reported method, with a slight modification [15,19]. Briefly, intestinal tissue was homogenized in ice-cold 1.15% KCl buffer. The samples were immediately centrifuged (10,000×*g* for 10 min) at 4°C to obtain a homogenate of intestinal mucosa. Aliquots of the homogenate were stored at −80°C until use.

2.8. Intracellular ROS measurement

Intracellular ROS generation was detected using the fluorescent probe, DCF-DA. DCF-DA readily diffuses through the cell membrane, where it is enzymatically hydrolyzed by intracellular esterases to form non-fluorescent DCFH. In the presence of ROS, DCFH is rapidly oxidized to form highly fluorescent DCF. The intensity of DCF fluorescence corresponds to the level of intracellular ROS formation. Following incubation, cells were collected and resuspended in PBS at a density of 10^6 cells/mL. An aliquot of the suspension (195 μL) was loaded into a 96-well plate, and 5 μL of DCFH-DA was added (final concentration 20 μM). The intensity of DCF fluorescence was detected over time using a FLUOstar Galaxy fluorescence plate reader (BMG Lab Technologies, Offenburg, Germany), with an excitation wavelength of 485nm and an emission wavelength of 530 nm.

2.9. Lactate dehydrogenase (LDH) analysis

The mucosal tissue was homogenized in KCl buffer, LDH leakage was assessed using a commercial kit (Sigma, St. Louis, MO, USA). The degree of LDH leakage reflects the level of tissue cytotoxicity.

2.10. Measurement of malondialdehyde (MDA)

The MDA concentration in rat mucosal samples was determined using the thiobarbituric acid method, with modifications [20]. The MDA concentration was normalized to the total protein concentration, and it is expressed as nmol/mg protein.

2.11. Measurement of GSH, the GSH/GSSG ratio, and total sulfhydryl groups (TSH)

The mucosal tissue was first homogenized in KCl buffer, and the levels of GSH and GSSG were measured using a glutathione assay kit (Cayman Chemical, Ann Arbor, MI, USA). The total amounts of GSH and GSSG were spectrophotometrically calculated by measuring the absorbance at 405nm using a microplate reader (Awareness Technology, Palm City, FL, USA). The activity of TSH was measured according to a previously reported method with some modifications [21].