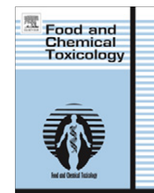
Food and Chemical Toxicology 53 (2013) 376–383

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

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



In vitro anti-inflammatory effect of apigenin in the *Helicobacter pylori*-infected gastric adenocarcinoma cells

Yuan-Chuen Wang *, Kai-Ming Huang

Department of Food Science and Biotechnology, National Chung Hsing University, 250 Kuo-Kuang Rd., Taichung 402, Taiwan, ROC

ARTICLE INFO

Article history:
Received 27 June 2012
Accepted 13 December 2012
Available online 22 December 2012

Keywords:
Apigenin
Oxidative burst
Helicobacter pylori
Inflammation
Gastric cancer

ABSTRACT

Infection with *Helicobacter pylori* causes extensive gastric epithelial cell inflammation which may progress to atrophic gastritis, intestinal metaplasia, and even gastric adenocarcinoma. Apigenin (4′,5,7-Trihydroxyflavone) is widely distributed in fruits and vegetables, and is a well-known antiinflammatory supplement with low cytotoxicity. In this study, we investigated the anti-inflammatory effects of apigenin in *H. pylori*-infected MKN45 cells, for which IκBα, cyclooxygenase-2 (COX-2), intercellular adhesion molecule-1 (ICAM-1), reactive oxygen species (ROS), interleukin-8 (IL-8), IL-6, IL-1β, and mucin-2 (MUC-2) expressions were examined. Apigenin treatments (9.3–74 μM) significantly increased the IκBα expression, and thus inhibited nuclear factor kappa B (NF-κB) activation, and the inflammatory factor (COX-2, ICAM-1, ROS, IL-6, and IL-8) expressions decreased. The ROS levels decreased partially based on the intrinsic scavenging property of apigenin. In summary, apigenin treatments effectively inhibited NF-κB activation and the related inflammatory factor expressions, as well as increased MUC-2 expression in the *H. pylori*-infected MKN45 cells. The compound shows great potential as a candidate agent for the inhibition of *H. pylori*-induced extensive gastric epithelial cell inflammation.

© 2012 Elsevier Ltd. All rights reserved.

1. Introduction

Infection with *Helicobacter pylori* is strongly associated with gastric cancer and gastric adenocarcinoma (Forman et al., 1991). The WHO classified *H. pylori* as a group I carcinogen in 1994 (Anonymous, 1994). The *H. pylori* virulence factors include vacuolating cytotoxin (VacA), cytotoxin associated antigen (CagA), *H. pylori* neutrophil activating protein (HP-NAP), urease, flagella, and lipopolysaccharide (LPS) (Covacci et al., 1999; Montecucco and Rappuoli, 2001). Once *H. pylori* adheres to the host's gastric epithelial cells, signal transduction is activated through those virulence factors, by which immune response (i.e., macrophage/dentritic cell cytokine induction) is immediately initiated and inflammatory mediators [i.e., interleukin-8 (IL-8), IL-6, intercellular adhesion molecule-1 (ICAM-1), cyclooxygenase-2 (COX-2), and reactive oxygen species (ROS)] are induced (D'Elios and Andersen, 2007, 2009; Montecucco and Rappuoli, 2001; Naito and Yoshikawa, 2002; Peek and Blaser, 2002; Peek et al., 2010).

H. pylori-induced cell signal transductions include nuclear factor kappa B (NF-κB), mitogen-activated protein kinase (MAPK), and 5-lipooxygenase (5-LOH) pathways, of which the NF-κB pathway plays a key role (Crantree and Naumann, 2006; Kim et al., 2010; Naito and Yoshikawa, 2002; Peek et al., 2010). Through toll-like receptor 4 (TLR4) and type IV secretion system (T4SS), *H. pylori* LPS and CagA respectively enter into host cells to activate NF-κB-inducing kinase (NIK) and IκB kinase α/β (IKKα/β). NF-κB is a dimer of proteins including p50, p52, p65, and cRel; which forms a trimer complex (mostly IκBα/p50/p65) with IκBα in cytoplasm (Jobin and Sartor, 2000; Jocobs Marc and Harrison Stephen, 1998). The IκBα degradation occurs after phosphorylation by IKKα/β, resulting in the NF-κB dimer (p50/p65) nucleus translocation and thereby the related gene (IL-8, IL-6, IL-1β, COX-2, ICAM-1, iNOS) transcriptions (Crantree and Naumann, 2006; Naito and Yoshikawa, 2002; Peek et al., 2010). MAPK activation is induced by CagA, which concerns three kinase cascades: C-terminal Jun-kinase (JNK); extracellular signal regulated kinase 1/2 (ERK1/2); and p38 kinase. The MAPK cascades lead to the activation of transcription factor activator protein 1 (AP-1), which regulates IL-8, NADPH oxidase 1 (Nox1), COX-2, and iNOS gene expressions (Bonizzi and Karin, 2004; Cho et al.,

Abbreviations: AP-1, activator protein 1; CagA, cytotoxin associated antigen; COX-2, cyclooxygenase-2; ERK1/2, extracellular signal regulated kinase 1/2; EtBr, ethidium bromide; H_2DCF-DA, dihydrodichlorofluorescein diacetate; HE, dihydroethidium; ICAM-1, intercellular adhesion molecule-1; IKKα/β, IκB kinase α/β; IL-8, interleukin-8; MUC-2, mucin-2; iNOS, inducible nitric oxide synthase; JNK, C-terminal Jun-kinase; LPS, lipopolysaccharide; 5-LOH, 5-lipooxygenase; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor kappa B; NIK, NF-κB-inducing kinase; Nox, NADPH oxidase; PGE2, prostaglandin E2; ROS, reactive oxygen species; TRL4, toll-like receptor 4; T4SS, type IV secrete system; VacA, vacuolating cytotoxin.

* Corresponding author. Tel.: +886 4 2284 0385x4220; fax: +886 4 2285 4053.
E-mail address: ycwang@nchu.edu.tw (Y.-C. Wang).

0278-6915/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.fct.2012.12.018

2010; Crantree and Naumann, 2006; Lambeth, 2004; Montecucco and Rappuoli, 2001; Naito and Yoshikawa, 2002; Peek et al., 2010).

Of the *H. pylori*-induced NF-κB/AP-1-dependent inflammatory factors, IL-8 plays an important role. IL-8 activates the CD11b/CD18 dimer which forms a complex with neutrophil, and then the complex activates ICAM-1 on the vascular endothelial cell membrane. That tetramer (CD11b/CD18/neutrophil/ICAM-1) infiltrates gastric epithelial cells and releases high amounts of ROS ($O_2^{\cdot-}$, H_2O_2, HOCl, OH·, and $^1O_2$) through neutrophil NADPH oxidase, resulting in oxidative burst (Kroemer et al., 1998; Montecucco and Rappuoli, 2001; Naito and Yoshikawa, 2002; Peek et al., 2010). Additionally, Nox1 activation is also a main source of ROS (Lambeth, 2004; Montecucco and Rappuoli, 2001). COX-2 is believed to be an important inflammatory contributor which metabolizes archidonic acid to prostaglandin E2 (PGE2), resulting in severe inflammation (Peek and Blaser, 2002). The *H. pylori*-induced oxidative stress, COX-2/PGE2, and proinflammatory cytokines cause extensive gastric epithelial cell inflammation which then progresses to atrophic gastritis, intestinal metaplasia, dysplasia, and finally gastric adenocarcinoma (Peek and Blaser, 2002).

Apigenin (Fig. 1), one of the most common flavonoids, is widely distributed in fruits and vegetables, especially abound in parsley, celery, guava, bilimbi fruit, garlic, and Chinese cabbage (Miean and Mohamed, 2001). The bioactivity of apigenin, including anticancer activity, anti-platelet aggregation, antioxidation, and anti-*H. pylori* activity, has been extensively studied (Das et al., 2010; Han et al., 2009; Wright et al., 2010; Yuan et al., 2007; Zhang et al., 2008). Furthermore, many papers have characterized apigenin's anti-inflammatory activity both *in vitro* and *in vivo* (Ha et al., 2008; Lee et al., 2007; Nicholas et al., 2007; Smolinski and Pestka, 2003). Apigenin is a well-known anti-inflammatory supplement with low cytotoxicity. However, there have been no reports on the anti-*H. pylori* induced inflammatory activity of apigenin. In this study, we investigated the anti-inflammatory effects of apigenin in *H. pylori*-infected gastric adenocarcinoma cells which exhibited IκBα, inflammatory factor (COX-2, ICAM-1, and ROS), chemokine (IL-8), cytokine (IL-6 and IL-1β), and mucin-2 (MUC-2) expressions. The anti-inflammatory mechanism of apigenin in the *H. pylori*-infected gastric adenocarcinoma cells is well established herein.

2. Materials and methods

2.1. Reagents

RNAase-free water, oligo dT primers, and dNTP were purchased from Bio Basic (Markham, Canada), with ethidium bromide (EtBr), polyacrylamide, and dihydroethidium (HE) from Sigma–Aldrich (Steinheim, Germany; St. Louis, MO, USA), apigenin (4′,5,7-Trihydroxyflavone, 98%), isopropanl, and phosphoric acid from Wako (Osaka, Japan), RNase inhibitor from Invitrogen (Grand Island, NY, USA), dithiothreito from Biovision (CA, USA), chloroform from Tedia (Fairfield, Ohio, USA), dihydrodichlorofluorescein diacetate (H_2DCF-DA) from Molecular Probes (Eugene, OR, USA), polyvinylidine fluoride (PVDF) membrane and chemiluminescene reagent from PerkinElmer Life Sciences (Waltham, MA, USA), anti-MUC-2 and anti-β-actin from Abcam (Cambridge, UK), anti-IκBα from Santa Cruz (Santa Cruz, CA, USA), anti-COX-2 and human enzyme-linked immunosorbent assay (ELISA) kits IL-1β, IL-6, IL-8, and ICAM-1 from R&D (Minneapolis, MN, USA), fetal bovine serum (FBS) from Gibco (Grand Island, NY, USA), Columbia agar from bioMérieux (Craponne, France), tryptic soy broth (TSB, pH 7.3) from Difco (Franklin Lakes, NJ, USA,), Rosewell Park Memorial Institute 1640 medium (RPMI 1640) from Gibco (NY, USA), REzol™ C&T from PROtech (Taipei, R.O.C.), PCR buffer from Fermentas (Burlington, Canada), agarose from PROtech (Solon, Ohio, USA), lysis buffer, transcriptase, and transcriptase reaction buffer from Promega Biotech (Madison, WI, USA), comassie brilliant blue G-250 from Amersco (Solon, Ohio, USA), peroxidase-conjugated goat anti-mouse IgG (H + L) from Jackson ImmunoResearch Laboratories (West Grove, PA, USA), and medical X-ray film from Fuji (Tokyo, Japan).

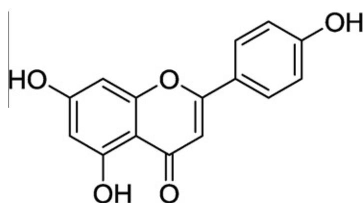


Fig. 1. Chemical structure of apigenin (4′,5,7-trihydroxyflavone). IL-8 production (pg/ml).

2.2. Cells and H. pylori strain

MKN45 cells (a human stomach cancer, poorly differentiated adenocarcinoma, JCRB0254) was obtained from the Japanese Collection of Research Bioresources (JCRB; Osaka, Japan). The cells were cultivated in RPMI 1640 supplemented with 10% FBS at 37 °C in an atmosphere of 5% CO_2.

H. pylori strain ATCC 700824 (*cagA*/*vacA* positive) was obtained from American Type Culture Collection (ATCC). A volume of 0.1 mL of *H. pylori* suspension was added in 5 mL TSB, with Columbia agar (pH 7.3) slant containing 5% (v/v) FBS formed at the bottom of the test tube. The broth was incubated in a microaerophilic jar system (BBL, Lawrence, Kansas, USA) presenting a gas composition of 5% O_2 and 10% CO_2-in-air [an OXOID (Thermo Scientific, Sunnyvale, CA, USA) BR 056A gas-generating kit was used for this purpose] at 37 °C for 72 h to produce 0.5–2.0 x 10^7 CFU/mL of the bacterial counts.

2.3. H. pylori infection and apigenin treatments

MKN45 cells were seeded onto a 96-well (1×10^6 cells/mL, 0.1 mL), 24-well (1×10^6 cells/mL, 1 mL), or 6-well (1×10^6 cells/mL, 2 mL) plate and cultivated at 37 °C in an atmosphere of 5% CO_2 for 24 h (80% confluence). After washing with PBS, the cell monolayers were treated with with 2-fold dilution apigenin and *H. pylori* ATCC 700824 (MOI = 1:70). The cell mixture was then incubated at 37 °C in an atmosphere of 5% CO_2 for 6 h [for mRNA (COX-2, ICAM-1, MUC-2, IL-8) expressions] or 24 h [for protein (COX-2, and MUC-2) expressions and cytokine/chemokine (IL-1β, IL-6, and IL-8), ICAM-1, and ROS levels]. Additionally, the cell mixture was incubated at an atmosphere of 5% CO_2 for 20 and 30 min for the IκBα mRNA and protein expressions, respectively. Non-apigenin treated cells inoculated with and without *H. pylori* ATCC700824 were used as the negative and cell only controls, respectively. The experiment was performed in triplicate.

2.4. Reverse transcription-polymerase chain reaction (RT-PCR) analysis

The mRNA (IL-8, COX-2, IκBα, MUC-2, and ICAM-1) expressions in the *H. pylori*-infected MKN45 cells were measured. Total RNA was extracted from the *H. pylori*-infected 6-well plate cells according to the manufacture's instruction, for which REzol™ C&T, chloroform, isopropanl, 75% ethanol, and RNAase-free water were used. The extracted RNA was quantified using a spectrophotometer (Hitachi U-1100, Tokyo, Japan) at 260 nm. The RNA was reverse transcribed into cDNA as follows: total RNA (5 μg, 6 μL), oligo dT primers (100 ng/μL, 5 μL), dNTP (10 mM, 1 μL), and RNAase-free water were incubated at 65 °C for 5 min and subsequently in an ice-bath for 1 min; and then the mixture was incubated with transcriptase reaction buffer (4 μL), RNase inhibitor (40 U/μL, 1 μL), transcriptase (1 μL), and dithiothreitol (1 M, 2 μL) at 37 °C for 1 h and subsequently at 70 °C for 15 min. The cDNA sample (1 μL) was amplified with forward and reverse primers (10 μM, 0.35 μL), DreamTaq™ DNA polymerase (5U/μL, 0.12 μL), dNTP (2.5 mM, 1.2 μL), PCR buffer (1.5 μL), and RNAase-free water (10.48 μL) using Thermal Cycler (MJ, Quebec, Canada). The primers were IL-8: 5′-GCTTTCTGATGGAAGAGAGC-3′ (forward) and 5′-GGCACAGTGGA ACAAGGACT-3′ (reverse) giving rise to a 566 bp product; COX-2: 5′-TTCAAATGA-GATTGTGGGAAAATTGCT-3′ (forward) and 5′-AGATCATCTCTGCCTGAGTATCTT-3′ (reverse) giving rise to a 254 bp product; IκBα: 5′-GATCAGCCCTCATTTTGTTGC-3′ (forward) and 5′-TAACTTTTCACCCCACATCAC-3′ (reverse) giving rise to a 305 bp product; MUC-2: 5′-CTTCGACGGACTCTACTACAGC-3′ (forward) and 5′- CTTTGGT GTTGTTGCCAAAC-3′ (reverse) giving rise to a 385 bp product; ICAM-1: 5′-CAA-GAACCTTACCCTACGCT-3′ (forward) and 5′-CACTGTCTGCAGTGTCTCCT-3′ (reverse) giving rise to a 532 bp product; β-actin: 5′-TCACCCACACTGTGCCCATCTACGA-3′ (forward) and 5′-CAGCGGAACCGCTCATTGCCAATGG-3′ (reverse) giving rise to a 295 bp product. The β-actin was used for normalization. The PCR amplification was performed as the following steps: the first cycle at 95 °C for 2 min, 25–30 repeat cycles of denaturation at 95 °C for 30 s, annealing at 65 °C (IL-8, COX-2, IκBα), 63 °C (ICAM-1), or 58 °C (MUC-2 and β-actin) for 30 s, and extension at 72 °C for 30 s (IL-8, COX-2, IκBα, MUC-2, and β-actin) or 2 min (ICAM-1); and the final cycle at 72 °C for 2 min. The PCR products were separated on 1.5% agarose gel, staining with 10 mg/mL EtBr, and visualized by UV transilumination. Band density was analyzed by Gel-Pro Analyzer software, version 4.0.00.001 (Bethesda, MD, USA). The experiment was performed in triplicate.

2.5. Western blot analysis

Protein (COX-2, IκBα, and MUC-2) expressions in the *H. pylori*-infected MKN45 cells were measured. The *H. pylori*-infected 6-well plate cells were washed with PBS, lysed with lysis buffer, and centrifuged at 4 °C and 12,000*g* for 15 min. The

Download English Version:

https://daneshyari.com/en/article/5851402

Download Persian Version:

https://daneshyari.com/article/5851402

Daneshyari.com